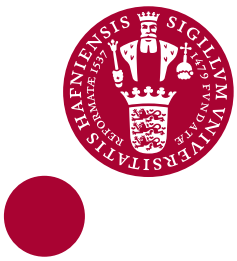

ANALYSIS OF JOINT EFFECTS BETWEEN BIOSECURITY, PRODUCTION, VACCINE AND ANTIMICROBIAL USE

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CONTENTS

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MULTIFACTORIAL ANALYSES

BAYESIAN NETWORKS STRUCTURE DISCOVERY: A TOOL TO CLARIFY ASSOCIATIONS BETWEEN HOUSING, MANAGEMENT AND WELFARE OF LAYING HENS

A. COMIN*, G. KRATZER, A. JEREMIASSON AND L. KEELING

SUMMARY

Bayesian network structure discovery is a form of graphical modelling based on machine learning that facilitates interpretation of complex biological systems. This multivariate approach was applied to outline the inter-relationships between information collected in an animal welfare control programme for laying hens in Sweden. The resulting directed acyclic graph identified the housing and management factors associated, or not, with the animal welfare indicators. Type of housing system, farm tidiness, management routines for manure removal and barn infrastructures were the key drivers of animal welfare. The main advantage of this approach was the holistic view, accounting for mutual dependence of all variables and highlighting both direct and indirect pathways to reach the same welfare goals. This opens a whole range of future work exploring which of the available pathways represent the most cost-effective intervention options, amongst other applications.

INTRODUCTION

In the European Union (EU), conventional battery cages had been the dominating housing system for laying hens until the official ban on January 1st 2012 (European Commission, 1999). In Sweden, however, housing of laying hens in battery cages was already banned in 1988, making it one of the first countries in the world to develop alternative systems. During the transition period from battery cages to alternative systems, a welfare control programme for laying hens was developed by the Swedish Egg Association, together with the Swedish Board of Agriculture and the Swedish University of Agricultural Sciences. The goal was to monitor and ensure that animal welfare was not negatively affected by the new housing systems. Currently, the program consists of a checklist of 31 control points evaluating facilities, barns, equipment, management and animals, and it is assessed every fourth year in each flock on a farm. However, a formal epidemiological evaluation of the data collected in this program investigating the impact of housing and management on animal welfare has not yet been carried out.

The welfare status of individuals or group of animals likely depends on many inter-related variables. Such complexity can be underestimated by traditional multivariable regression models, such as linear or generalized linear models, where one variable is designated as a single

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response variable and all the remaining variables as predictors. It has therefore been suggested that a holistic, multidimensional approach is preferable when conducting epidemiological analyses of complex biological data (Lewis & Ward, 2013). Bayesian network (BN) modelling is a modern graphical approach for analysing complex systems and is increasingly finding application in areas such as genetics, systems biology and epidemiology (Jansen et al., 2003; Poon et al., 2007; Ward & Lewis, 2013; Firestone et al., 2014) thanks to its ability to generalise standard regression methods. The focus of BN modelling is on structure discovery: determining an optimal statistical model (i.e. graphical structure) directly from observed data, allowing all variables to be potentially mutually dependent. It further allows discrimination between indirect and direct associations, by estimating the joint probability distribution of all variables of interest (Lewis et al., 2011).

The objective of this research was to apply BN structure discovery to clarify the associations between housing system, rearing facilities, farm management and welfare indicators in laying hens. The goal was to differentiate variables that are directly associated with poor welfare status of the birds, and therefore promising targets for intervention, from variables that are only indirectly associated with the welfare status, and can therefore affect it through their association with other variables.

MATERIALS AND METHODS

Data source

Data were collected through the industry-based welfare control programme from 2010 to 2014 in 397 flocks, belonging to 193 different farms affiliated to the Swedish Egg Association (i.e. 65% of all the commercial layer farms in Sweden). During the welfare inspections, 31 different checkpoints were scored from 0 (worse) to 4 (best) to evaluate the status of the flock in terms of housing facilities, structure of the barns, farm equipment, general management and animal conditions. For this analysis, the scores were dichotomized. Scores equal to 3 or 4 were considered *good* and kept as the baseline reference group, while those between 0 and 2 were considered *bad* and set as the outcome. Of the 31 available, 11 checkpoints were included in the analysis. The selected checkpoints were either linked to animal conditions or to the Swedish welfare legislation and had enough variation to provide some statistical insight (i.e. the frequency of outcomes for a checkpoint should have been at least 0.025). Additional information, beyond the scored checkpoints, included whether the barn belonged to organic production, flock size (i.e. number of birds), age of the flock (in weeks), mean monthly mortality, and type of housing system (i.e. enriched cage, single-tier system, multi-tier system). The latter was subsequently coded as a set of two dummy variables (i.e. enriched cage: yes/no and single-tier: yes/no) to be handled by the model. In addition, the variables age of the flock and mean monthly mortality, which were highly skewed, were transformed (by taking binary logarithm and the square root, respectively) to facilitate the Bayesian estimation of the marginal distributions. In total, 17 variables (14 binary and 3 continuous) related to housing (4), facilities and management (9), and animals (4) were included in the analysis (Table 1).

Bayesian networks

An additive Bayesian network modelling approach (ABN) was applied. ABN models are a special type of BN models which can be seen as directly analogous to multivariate generalized linear regression, where each variable in the data are modelled by an additive multivariable regression model. All BN models are comprised of two reciprocally dependent parts: the

structure and the parameters. The model structure is defined by a directed acyclic graph (DAG), which is the graphical representation of the factorisation of the joint probability distribution of all random variables. Each node of a DAG represents a random variable, while arcs represent probabilistic dependencies between them. The arcs' direction gives the flow of information, and the model parameters represent the quantification of the relations showed by the arcs. Incoming arrows to a node and regression coefficients encode the way the index node is predicted based on its parent set, analogously to an additive multivariable regression model. The model applied in this study uses a Bayesian approach for both structure discovery and parameter learning, and as such, relies on prior information. A uniform structural prior (i.e. all eligible DAG structures were equally supported in the absence of any data) and non-informative priors for all the parameters at each node were used.

A three-step procedure was used to determine an optimal ABN model for the data: (1) identification of the single best model (i.e. the DAG with optimal goodness of fit to the available data); (2) adjustment for overfitting; (3) addressing potential data clustering. The optimal model was identified by an exhaustive (exact) search of the data (Koivisto & Sood, 2004), iterated across incremental parent limits, accounting for existing knowledge about data structure that could guide the search. Such prior information about causality was included by banning some specific arcs from being considered in the final DAG. The modelling was carried out using the software R (R core team, 2017) and the package 'abn' (version 1.0.2) (Kratzer et al., 2016).

The best DAG (i.e. output of step 1) was subsequently adjusted for overfitting by parametric bootstrapping using Markov chain Monte Carlo (MCMC) simulations, as described in Lewis and McCormick (2012). Arcs which were not recovered in at least 50% of the bootstrap results were removed, to ensure the generalizability of the model to unseen data (in analogy to major consensus trees in phylogenetics, Weiß & Göker, 2011). Parametric bootstrapping was performed using the software JAGS (Plummer, 2003) through the R package 'rjags' (version 4.3.0) (Plummer, 2016).

The final step in the structure discovery procedure was to address potential within-group correlations. Flocks of hens from the same farm are potentially correlated as they are present within the same farm environment and likely subject to the same management processes. Not explicitly accounting for such within-group clustering might result in an underestimation of the variance, and therefore unreliable parameter estimates. The usual solution to overcome this problem is to move from generalized linear models (GLM) to generalized linear mixed models (GLMM), which include random effects at group level to incorporate additional variance into the sampling distributions. Therefore, the method applied by McCormick et al. (2013) was adopted, and the final pruned DAG (i.e. output of step 2) was fitted to the original data by means of MCMC simulations (using JAGS), including a random effect at farm level for each node. The resulting new marginal densities (output of 20 000 MCMC samples from two chains with different starting values, after a burn-in of 5000 iterations per chain and a sampling lag of 50 to avoid autocorrelation) were then compared to those previously obtained, to see whether they become wider due to the clustering effect. Any arcs that were no longer statistically supported (i.e. the 95% credible intervals of the marginals crossed the origin) were further removed to produce a final DAG adjusted for clustering effect.

Strength of associations

The strength of associations among variables is as important as the existence of such associations, and it is a complementary metric to regression coefficients. Given that the

classical metric to account for significance, the p-value, is essentially not applicable here, a link strength metric called *true average link strength percentage* (LS%) was calculated (Ebert-Uphoff, 2009). LS% expresses the number of percentage points the uncertainty in the outcome Y is reduced by knowing the state of the predictor X, if the states of all other parent variables are known (averaged over the parent states using their actual joint probability).

RESULTS

The optimal DAG had a maximum marginal likelihood of -3394 with a parent limit of 3 and a total of 23 arcs. After adjustment for overfitting, two arcs were pruned (i.e. going from flock size to inner biosecurity and from flock size to alarm) resulting in a final DAG with 17 nodes and 21 arcs (Fig. 1). Rectangles indicate binary nodes and ovals represent continuous nodes. Solid arrows represent a positive association and dashed arrows a negative association. The width of the arrows reflects the strength of the association between two nodes. Animal-related variables are coloured in black; housing-related variables are given in grey; variables related to facilities and management are coloured in white.

The scope of the present study was to identify direct and indirect associations between housing/managerial factors and welfare of laying hens. Therefore, the inference was focused on three animal-based welfare indicators: feather condition, presence of external parasites (mites) and flock mortality. In addition, two environment-based welfare indicators – lighting and air quality – were inspected, both of which are known to have a strong impact on production as well as being tightly linked to welfare legislation.

The parameters estimated from the final pruned DAG after accounting for potential clustering within farm are reported in Table 2, together with the estimated link strength for the arcs. The parameters have the usual interpretation as posterior marginal log odds ratios for binary variables and correlations for continuous ones. Log odds ratios were exponentiated to obtain the odds ratios (OR). For transformed data (i.e. square root of mortality), the coefficients were subsequently transformed to make inference in the original scale as the amount of change of the response given a predictor. Adjustment for farm clustering did not suggest any further need for arc pruning, but it produced slightly wider credible intervals for some of the marginal estimates.

Flock mortality was directly associated with housing animals in enriched cages, which had lower mortality rates compared to multi-tier system barns. The value of -0.62 for the correlation between square root of mortality and enriched cages (Table 2) translates into a reduction of mortality of -0.023% when moving from multi-tier barns (i.e. the baseline reference) to enriched cages. Another factor affecting mortality, although the relation was quite weak (link strength = 2.7%), was the age of the flock. For every week increase in age, there was an associated 0.0006% increase in mortality (corresponding to 0.21 correlation in the transformed scale, Table 2). In addition, age was the only factor affecting feather condition, which was worse in older hens. According to the estimated parameters, the risk of poor feather condition increased by 68% for every week increase in age (OR=1.68, Table 2).

Table 1. Variables included in the model. Welfare-related variables are marked in bold.

Level	Variable	Description	Levels / Unit of measure
Housing	Organic	Organic egg production	0 (no), 1 (yes)
	Enriched cage[a]	Animals are housed in enriched cages	0 (no), 1 (yes)
	Single tier[a]	Animals are housed in single-tier barns	0 (no), 1 (yes)
	Flock size	Number of birds housed in the flock at the beginning of the production cycle	Number of birds
Facilities and management	Outer biosecurity	Measures to prevent rodents or wild birds from entering the barn. Secured ventilation, no holes in the walls, zone without vegetation outside the barn.	0 (good), 1 (bad)
	Packing room	This room must be clean and in order since eggs, which are considered food, are handled here. The walls must be painted, so that they can be easily cleaned, and the floor in good condition. There must be hot/cold water and a visitor journal.	0 (good), 1 (bad)
	Inner biosecurity	The barn must have an external biosecurity zone, with a barrier of at least 40 cm that you must step over after you have changed your shoes and taken off your jacket. There also must be an internal biosecurity zone at each barn entrance, also with a barrier of at least 40 cm. Here you must change shoes again and put on protective clothing.	0 (good), 1 (bad)
	Lighting	The quality of the lighting conditions. Does the barn have windows? Are the windows used? How are the windows regulated, automatically or manually? What is the quality of lamps?	0 (good), 1 (bad)
	Air quality	Air quality in the barn. Maximum level NH_3 =10 ppm in cages and multi-tier or maximum level NH_3= 25 ppm in single-tier. Maximum CO_2= 3000 ppm.	0 (good), 1 (bad)
	Water	Are there enough drinking places? Has the water been tested yearly? Are the drinking lines regularly flushed	0 (good), 1 (bad)
	Furnishing & litter	The amount of floor eggs in single-or multi-tier systems. The quality of the litter on the floor in single- or multi-tier systems. The litter must be dry and friable, not wet and hard. In enriched cages there must be enough litter in the litter bath.	0 (good), 1 (bad)
	Alarm	If the barn houses more than 2000 birds, it is required by law to have an alarm system. The alarm should be connected to ventilation, electricity, temperature and water.	0 (good), 1 (bad)
	Logbook	The farmer should note amount of collected eggs, number of culled birds and vaccination routines. The barn should be approved in both the voluntary Salmonella control programme and the round worm control programme.	0 (good), 1 (bad)
Animals	**Feather condition**	Quality of plumage. No featherless areas.	0 (good), 1 (bad)
	Mites	Occurrence of red mite infestations and management routines	0 (good), 1 (bad)
	Age of the flock	Age of the flock since the onset of production cycle	Weeks
	Mean monthly mortality	Cumulative proportion of dead birds in the flock at inspection divided by number months since the onset of production cycle	Percentage

[a]When both enriched cages and single-tier equals 0, it means that animals are housed in multi-tier barns (which represents the reference category in the original multinomial variable).

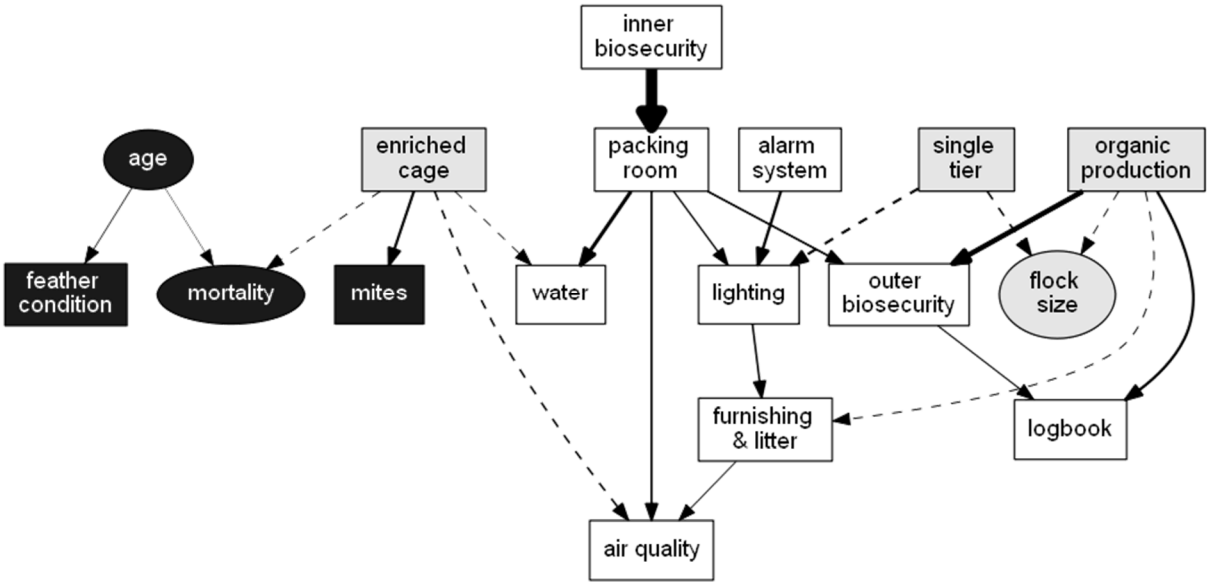

Fig. 1 Final globally optimal DAG of housing facilities, farm management and welfare indicators in laying hens (n = 397).

Table 2. Estimates of the marginal posterior densities (median and 95% credible interval) and link strength for the welfare-related variables included in the model, after accounting for herd clustering.

Parameter	Marginal density	95% CI	Interpretation	Link strength
√Mortality | enriched cage	-0.62	[-0.89; -0.35]	Correlation	5.2%
√Mortality | age	0.21	[0.12; 0.29]	Correlation	2.7%
Feather condition | age	1.68	[1.30; 2.21]	Odds ratio	4.3%
Mites | enriched cage	8.06	[2.94; 23.52]	Odds ratio	11.3%
Lighting | single tier	0.02	[0.00; 0.16]	Odds ratio	11.0%
Lighting | packing room	33.46	[3.39; 401]	Odds ratio	7.1%
Lighting | alarm	55.39	[9.23; 493]	Odds ratio	11.8%
Air quality | enriched cage	0.00	[0.00; 0.03]	Odds ratio	8.1%
Air quality | packing room	25.78	[5.90; 139]	Odds ratio	9.0%
Air quality | furnishing & litter	9.36	[2.27; 35.84]	Odds ratio	5.0%
Furnishing & litter | lighting	12.70	[3.10; 517]	Odds ratio	8.2%

The presence of external parasites was more often associated with enriched cages, which were 8.1 times as likely as multi-tier systems to house birds with moderate to severe mite infestations. On the other hand, enriched cages were associated with a better score regarding air quality compared to multi-tier systems, although the binomial regression failed to provide an odds ratio due to data separation (i.e. there were no flocks housed in enriched cages scoring badly for air quality).

Air quality in the barns was negatively affected by poor litter quality (OR=9.3), which in turn was related to improper lighting conditions (OR=12.7), although the estimates of the marginal posterior densities were associated to quite wide credible intervals (due to only few flocks scoring badly for such checkpoints). Lighting was better in barns with single-tier systems, which had 98% less chance of having improper lighting conditions compared to multi-tier systems (OR=0.02). Risk factors for poor lighting were improper alarm systems (OR=55) and untidy packing room (OR=26), which were however associated to wide credible intervals.

Interestingly, none of the five considered welfare indicators were directly affected by production type, suggesting that animal welfare did not differ between conventional and organic egg production for the flocks included in this study.

DISCUSSION

This study explored the complexity of welfare determinants in laying hens by investigating the inter-relationship between housing system, facilities, management and welfare indicators. For this purpose, a machine learning procedure referred to as structure discovery was applied to data collected through the welfare control programme in Sweden.

Of the animal-based welfare indicators included in this study, only feather condition could potentially represent a concern for Swedish farmers (scoring badly in 21% of the inspected flocks, data not shown), since severe mite infestations were rare (4% of inspected flocks) and mortality was far below the acceptable threshold (i.e. < 0.5%). On the other hand, feather condition seemed only to be affected by the ageing of the flock. This may not be so surprising itself, since the plumage is likely to become more worn as the hen gets older. What, however, was interesting was the lack of further associations between feather condition and any other variable included in the model. This differs from the knowledge reported in the literature where flocks with better feather condition have also been shown to have a lower mortality (Heerkens et al., 2015). The holistic approach offered by ABN permitted accounting for the potential dependency among all the variables included in the model showing that, for that particular set of variables and data, mortality was conditionally independent from feather condition given age (i.e. edges going from age to feather condition and to mortality, d-separating them). This means that once the age of the flock is known, then knowing the status of feather condition will not change the belief about flock mortality. In more epidemiological terms, age is a confounder for the relationship between feather condition and mortality and once accounted for, the association between the two disappears.

The occurrence of mites and flock mortality were both directly linked to enriched cages, which represented a risk factor and a protective factor, respectively. A study by Höglund et al. (1995) showed that red mites were less prevalent among layers housed in battery cages compared to alternative systems, but there are currently no studies comparing the occurrence of red mites in enriched cages and loose housing systems. The significantly lower flock mortality observed in the enriched cages could be due to lower bacterial and parasitic pressure compared to multi-tier systems. In fact, it has been reported that the occurrence of bacterial and parasitic diseases is higher in Swedish loose housing systems both with and without outdoor access (Fossum et al. 2009).

The three animal-based welfare indicators were only linked to animal-related or housing-related variables (i.e. age and enriched cages). This suggests that none of the variables associated with facilities and management included in the model are likely to be useful targets

for intervention to improve feather condition, mite infestations and flock mortality. It may also indicate that the current animal-based welfare indicators are already the best they can be and cannot be improved by intervening in any of the farm management variables considered in the analysis.

The environment-based welfare indicators included in this study scored badly in 5 and 8% of the evaluated barns – for lighting and air quality, respectively – and showed a complex inter-relationship with several managerial and housing variables. The two indicators were conditionally independent and yet indirectly linked to each other through multiple pathways. For example, one path went from lighting to air quality through the intermediate node furnishing and litter, indicating that air quality in the barn was negatively affected by poor litter quality, which in turn was directly related to improper lighting conditions. The direct association between poor litter quality and poor air quality was expected, since ammonia, which affects air quality, is produced from uric acid by microbial enzymes and degradation in the manure (David et al., 2015). As for the relationship between improper lighting condition and poor litter quality, it can be speculated that it reflects older barns with poor housing facilities. Another interesting chain connecting air quality and lighting was through their common ancestor: packing room. This might suggest that less meticulous management routines (such as having an untidy packing room) are more likely to perpetuate within a farm, producing unsatisfactory environmental conditions for the welfare of the layers. As a consequence, improving the facilities (e.g. newer barns with better lighting) and/or the management routines (e.g. careful removal of litter and manure) have the potential to positively impact the environmental-based welfare indicators. An interesting follow-up of this study could therefore be the formal evaluation of which of the alternative paths represents the most cost-effective target for intervention to improve animal welfare.

ABN modelling outlined graphically the underlying (but unknown) process that most likely generated the observed data. It compactly represented the joint probability distribution for a multivariate domain by using a DAG to encode conditional independences. Despite the incoming arcs to a node indicating statistical relationships and not causal statements (exactly as it happens for statistically significant coefficients in traditional regression), incorporating additional expert knowledge to guide the search of the optimal model was a step towards the identification of causal mechanisms. Incorporating additional causal knowledge in the form of network restrictions – as was done here by banning the arcs that were known to be unrealistic from a biological point of view – has also been demonstrated to enhance the search strategy, leading to improved network structures in less time (De Campos & Castellano, 2005).

Despite being computationally demanding (i.e. exact structure discovery is only feasible for around 20 variables in the current setting), ABN modelling offered a richer tool for statistical inference than other regression-based modelling, as it represented a direct generalisation of GLM/GLMM to multiple dimensions. Its multivariate approach made it possible to account for non-independence between explanatory variables, which is a key consideration when analysing observational study data. The resulting DAG outlined housing and management factors that were directly associated to animal welfare and which could potentially become targets for intervention. Furthermore, it outlined alternative indirect pathways to reach the same welfare targets, which might be worth further investigation to identify the most cost-effective intervention options.

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ANALYSIS OF JOINT EFFECTS BETWEEN BIOSECURITY, PRODUCTION, VACCINE AND ANTIMICROBIAL USE

A.B. KRUSE*, L.R. NIELSEN AND L. ALBAN

SUMMARY

It is important to identify ways of maintaining high productivity in pig production while keeping antimicrobial use low. The aim of this study was to explore the joint effects between biosecurity, production, vaccine and antimicrobial use. A factor analysis was conducted on biosecurity data collected via computer-assisted telephone interviews and data on a production parameter, purchased vaccines and antimicrobials prescribed in 152 Danish sow herds. Four factors were identified. Factor 1 implied herd type, productivity and antimicrobial use. Factor 2 covered biosecurity. Factor 3 represented focus on foreign employees, cross-fostering and export. Lastly, Factor 4 covered health status and vaccine use. Productivity was positively correlated with antimicrobial use in Factor 1 but did not correlate with vaccination or biosecurity. Thus, when considering future pig production without zinc oxide and a continued focus on reducing antimicrobial use, it is important to consider alternative ways of keeping up a sustainable production.

INTRODUCTION

Pig producers in Denmark and the rest of the European Union (EU) are currently facing two major challenges related to the use of antimicrobials, whilst keeping up with production demands. Firstly, there is an increasing focus on how to decrease antimicrobial (AM) use. In Denmark, several interventions have been implemented over the last two decades, forcing pig producers to reduce their AM-use (DANMAP, 2016). Secondly, in June 2017, the EU Commission decided to phase out the use of zinc oxide within the proceeding 5-year period (European Commission, 2017). In Danish pig production, zinc oxide has been an important additive in the process of reducing AM-use for treatment of diarrhoea (DANMAP, 2016). Denmark has one of the lowest levels of AM-use per produced pig among EU Member States with a large pig production (ESVAC, 2017). The total AM-use measured in kilo active compound for pigs in Denmark has decreased by 16 % since the introduction of 'the Yellow Card scheme' in 2010. In the scheme, the veterinary authorities set limits on the quantity of AMs that can be prescribed to pigs (measured in Animal Daily Doses (ADD) per 100 animals per day) in each of three age groups (Sows including piglets, weaners from 7-30 kilo and finishers >30 kilo). The evaluation of antimicrobial prescriptions based on a 9-month rolling interval occurs for all pig herds in Denmark. Since 2010, the acceptable limit for AM prescriptions in pig production has been lowered three times (DANMAP, 2017).

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It is challenging for most farmers to reduce the AM-use further within the existing production system, especially in the future without access to zinc oxide. Besides a low AM-use, Danish pig production is characterised by high standards of biosecurity due to the well-established Specific Pathogen Free (SPF) system, moderate use of vaccines and a high level of productivity attributed to intensive breeding according to specific traits and good herd management practices. Biosecurity and appropriate use of vaccines are considered important disease prevention strategies that may be used to reduce the need for treatment (Postma et al., 2015). However, non-targeted use of vaccines may not lead to a lower AM use at a population level as shown by Temtem et al. (2016) and Kruse et al. (2017). Therefore, there is a need for further studies regarding the role of vaccines and biosecurity in relation to optimal AM-use. Moreover, biosecurity is considered critical to minimizing the risk of introduction of exotic diseases, such as African swine fever, which is currently spreading across Russia and Eastern Europe (EFSA, 2014). Finally, productivity is a constraining issue, since a farmer with a low productivity may not remain in business for very long.

The important question was how to fulfil the requirements from the authorities regarding limited AM-use without jeopardizing productivity. It was challenging to investigate, because many of the variables that can describe biosecurity, productivity, vaccination and AM-use in pig herds were expected to be highly correlated. Hence, multivariate statistical methods were relevant for these types of research questions. Therefore, the aim of this study was to explore the joint effects between AM prescriptions, biosecurity, productivity and vaccination in the Danish pig production using factor analysis. Furthermore, underlying interdependencies revealed in the factor analysis were used to identify sow herd typologies, and through these create feasible herd health strategies for the farmers and herd health advisors.

MATERIALS AND METHODS

Herd inclusion criteria

Conventional sow herds were identified using data extracted from the Danish Central Husbandry Register (CHR). The following criteria had to be fulfilled for a herd to be included in the study: The herd had productivity data collected for the yearly statistics on productivity in Danish pig herds in 2014 (SEGES, 2015), a minimum of 100 sows recorded in CHR and registration of weaners with a minimum of one pen place recorded in CHR. Some of the herds selected based on these criteria also had finishers registered, though this was not a criterion for enrolment. A variable called TYPE was created based on the number of weaner and finisher pen places compared to the number of sows for each herd. Two types of herds were identified and categorized according to the following criteria: 1) Farrow-to-finisher herds with numbers of weaner and finisher pen places of at least 1.5 times the number of sows representing herds with a substantial fraction of produced weaners kept until slaughter, 2) Farrow-to-weaner herds with numbers of weaner and finisher pen places of less than 1.5 times the number of sows, representing herds with no or only a small fraction of produced weaners kept until slaughter. The numbers of animals recorded for the sows and weaners age groups were included as two separate variables in the factor analysis together with the variable TYPE.

Biosecurity data

Biocheck.Ugent Pig® (Biocheck) is an online questionnaire and scoring system, which scores the level of biosecurity in pig herds, based on the answers given by the pig producer. Besides Denmark, Biocheck has previously been used to score the level of biosecurity in pig

herds in many other countries, e.g. Belgium, France, Germany, Netherlands and Sweden (Filippitzi et al., 2017). For this project, the English version of the questionnaire was translated into Danish. A few phrasings were changed to fit Danish conditions (10 questions) and selected country-specific questions were added (16 questions). The latter consisted mainly of questions related to foreign employees, washing of livestock trucks, transport and export of animals. The full questionnaire including 144 questions in 10 subcategories was used as the basis for a computer-assisted telephone interview, where the interviewers recorded the answers based on the pig producer's response to semi-open questions. Telephone interviews were conducted from August to December 2015 by three interviewers. To streamline the understanding of each question, all interviewers prepared for the interviews by going through the questionnaire thoroughly together and by visiting a farm to make sure they understood and agreed on the context behind every question. Scores for each subcategory, as well as for total external and internal biosecurity were automatically calculated by adding the answers from the Biocheck questions to the online version of the scoring system (http://www.biocheck.ugent.be/). Calculation of scores are based on a pre-weighting of each question and subcategory. The scores obtained range from 0-100, which illustrate the estimated importance for the introduction and spread of infectious diseases. Hence, a score of 0 corresponds to "total absence of biosecurity" and a score of 100 corresponds to "perfect biosecurity" according to the measures addressed in Biocheck (Laanen et al., 2013).

The answers to the questions from the adjusted questionnaire in Danish, including the additional questions, were included as data in the factor analysis. The questionnaire is available in Danish and English from the first author upon request.

Herd health and productivity data

Herd health and productivity data were provided by the Danish farm advisory services 'SEGES Pig Research Centre' (SEGES) upon permission from the farmer. Regarding productivity, the variable weaned piglets per sow per year, WEANERS PER SOW, was included. This variable indicates the overall productivity of the farrowing unit of a herd and is influenced by number of live born piglets, piglet and sow mortality as well as the performance of the sows in terms of farrowing percent. It is the measure most often used, when reporting productivity in sow herds in Denmark.

Status of enrolment in the SPF system in 2014 were included in the factor analysis. This represented herd health status and possibly biosecurity status, with the main emphasis in the SPF system being on external biosecurity.

Antimicrobial and vaccination data

Prescription of AMs and purchase of vaccines for Danish pig herds are recorded in the Danish Veterinary Medicines Statistics Program (VetStat). Data used in this study consisted of raw historical data from the VetStat database, retrieved on 1 June 2015.

The total amount of AMs prescribed in 2014 was extracted for each herd enrolled and used as a proxy for the AM-use. The prescriptions were divided into prescriptions for sows (including piglets) and weaners. Each prescription was converted into a number of ADD using standardized doses per AM product developed by the Danish Veterinary and Food administration. The ADD assumes a standard average weight of a sow (200 kg including piglets) and a weaner pig (15 kg) as well as the total amount and standard dose of the AM prescribed. The numbers of animals registered in CHR were used in the calculation of the

average ADD per 100 sows per day and of ADD per 100 weaners per day, respectively. These two variables were also included in the factor analysis.

Purchase data of all vaccine products against five disease agents were extracted from each herd in 2014. The five disease agents were *Mycoplasma hyopneumoniae* (MYC), *Actinobacillus pleuropneumoniae* (APP), Porcine Reproductive and Respiratory Syndrome (PRRS) Virus, Porcine Circovirus Type II (PCV2) and *Lawsonia intracellularis* (LAW). For a herd to be classified as using vaccines against one of these diseases, the herd had to have purchased the given vaccine(s) throughout the 2014 year. Individual categorical variables representing each of the five groups of vaccines were created and included in the factor analysis. These variables indicated whether the individual herd was using the vaccine in question or not.

Statistical analysis

All data management and descriptive statistics were carried out using the software R version 3.1.3. Factor analysis was conducted in SAS version 9.2. This technique requires observations for all variables included. In total, 80 variables had to be excluded, because of missing observations. The final data set contained both categorical and continuous variables, which required a scaling of the data prior to the factor analysis using the PROC PRINQUAL procedure in SAS. The procedure PROC FACTOR with orthogonal rotation (VARIMAX) was used on the transformed data. The Kaiser-Meyer-Olkin (KMO) measurement of sampling adequacy in the output was evaluated, and 30 variables each with KMO < 0.4 were excluded to increase the overall KMO. A scree plot illustrating the distribution of the eigenvalues was evaluated to determine the number of factors to include by identifying the break point between large and small eigenvalues, and ensuring that all Eigenvalues were above 1. For model diagnostics, the residual correlation matrix as well as the overall root-mean-square off-diagonal residual (RMSR) was inspected (Sharma, 1996). Variables with factor loadings ≥ 0.30 or ≤ -0.30 were included as being influential variables for a factor (Table 1). Variables with loading ≥ 0.25 or ≤ -0.25 were studied and considered as borderline effects and were included in a factor if the variable was not already represented with a higher score in one of the other factors. The interpretation of a factor was based on results from the five herds scoring highest and five herds scoring lowest on the respective factor.

The results from the factor analysis were further explored by plotting all herds according to their factor scores for two factors at a time. This enabled the authors to identify different herd typologies in collaboration with two Danish pig veterinarians. The herds were divided into four quadrants for each plot. The herds in each quadrant represent a specific type of farmer/farm, illustrated by a label showing the main characteristics and number of herds in that specific quadrant. The characteristics were based on the interpretation of the individual factors, and whether the herds in the specific quadrant scored high and/or low on the two factors in question. Moreover, ideas for how to move herds from a quadrant with a poor practice to a quadrant with a better practice were identified (e.g. from low to high productivity, biosecurity or AM-use level). To illustrate any difference between SPF and non-SPF herds, these two groups were marked in the factor plots, using the 'ellipse' function in R covering 95% of the observations in each group (Fig. 2 and Fig. 3).

RESULTS

Descriptive statistics

In total, 364 sow herds were initially selected based on the herd enrolment criteria. At least three attempts were made at different times of the day to reach all 364 by phone. It was not possible to get in contact with the farmers from 88 (24%) of these herds within the questionnaire data collection period. For 100 herds (28%), the farmer refused to participate. Sixteen farmers agreed to participate, but it was impossible to finalise interviews within the data collection period. In total, 160 interviews were finalised. Out of the number of farmers that were contacted (N=276), 58% were finally interviewed.

The scores from Biocheck revealed a higher external biosecurity level (Mean = 86, Min = 67, Max = 96) compared to the internal biosecurity level (Mean = 67, Min = 48, Max = 90) among the 160 herds.

Because there was no requirement regarding the number of weaners in each herd, it was revealed that some herds had too few weaners recorded to have a real on-going production of weaners. Because of this, some of the herds did not have any AM prescriptions for the weaner age group and these observations (n=8) were excluded from the dataset to be able to include this variable in the factor analysis. Therefore, the following results is based on data from the 152 herds with AM prescriptions for weaners. Out of these herds, 129 were enrolled in SPF. In total, 38 herds kept more than 1/3 of their piglets until slaughter and were therefore considered farrow-to-finisher herds. The mean herd size was 559 sows per herd. Vaccination against PCV2 and MYC was most frequently used (76 % and 59 % of the herds, respectively). Vaccination against APP was less frequently used (16% of the herds). Finally, only 13% of the herds were vaccinated against PRRS and LAW. The mean herd productivity, measured as WEANERS PER SOW was 30.8. The mean AM-use for sows and piglets was 2.3 ADD/100 animals/day and for weaners it was 10.2 ADD/100 animals/day.

Factor analysis

By evaluating the scree plot and Eigenvalues > 1, four factors were retained. The final factor analysis resulted in a RMSR value of 0.066. This is close to 0.05, which is recommended by Sharma (1996). The eigenvalues for each factor were: Factor 1 = 4.08, Factor 2 = 2.78, Factor 3 = 2.52 and Factor 4 = 2.30. In total, the four factors explained 27 % of the variance; Factors 1–4 contributed 9.5%, 6.5%, 5.9% and 5.4%, respectively. Based on the factor loading criterion, between seven and 11 variables were included in each factor as shown in Table 1.

Evaluating data from the five herds with the lowest and five herds with the highest factor loadings on the respective factor revealed the following explanations for the four factors:

- Factor 1 = Type of herd, productivity and AM-use: A herd scoring high on Factor 1 was typically a large farrow-to-weaner herd with newer facilities, high productivity, higher AM-use for sows and piglets and a higher AM-use for weaners.
- Factor 2 = General biosecurity: A herd scoring high on Factor 2 was a herd with several measures of importance for internal and external biosecurity in place.
- Factor 3 = Foreign employees, cross-fostering and export: A herd scoring high on Factor 3 could generally be characterised as having no export of piglets, and said they tried to prevent employees feeding pigs food leftovers and bringing food from foreign

countries. They also made much use of cross-fostering of piglets, had empty transport vehicles picking up sows for slaughter and said they had problems with rodents.

- Factor 4 = SPF and vaccination: A herd scoring high on Factor 4 was typically not enrolled in SPF and used several vaccines. Also, the transport vehicle was backed up to the stable when loading sows from these herds.

To characterise a herd scoring low on each of these factors, the negation should be used; for Factor 1 this would be a small farrow-to-finisher herd with old facilities, low productivity and low AM-use.

For identification of herd typologies, only factor plots of Factor 1 vs. Factor 2 (Fig. 2) and Factor 1 vs. Factor 4 (Fig. 3) were evaluated. Together, these two plots covered herd type, productivity and AM-use (Factor 1) vs. biosecurity (Factor 2) and vaccine use (Factor 4).

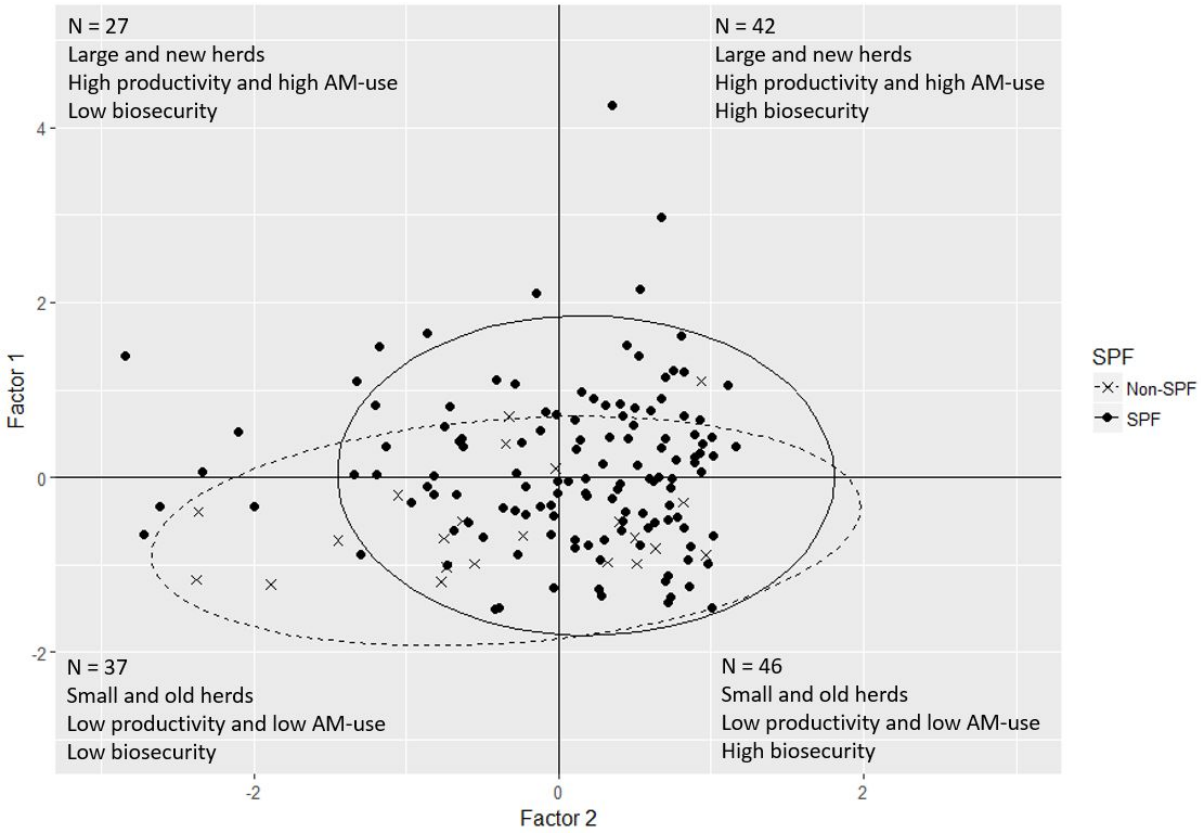

Fig. 2 Factor plot for Factor 1 vs. Factor 2 from the final factor analysis performed on data from 2014 and 2015 representing 152 Danish sow herds. Each point represents an individual herd located according to its scores on the two factors. In each of the four quadrants, the number of observations and an interpretation of the factors scores are given. SPF herds are marked with black dots, and the ellipse with bold line covers 95 % of the observations in this group. Non-SPF herds are marked with crosses, and the ellipse with dotted line covers 95 % of the observations in this group.

Table 1. Factor loadings for variables included in the final factor analysis on data from 2014 and 2015 covering 152 Danish sow herds. Factor loadings above 0.30 and lower than -0.30 are marked in bold, indicating the variables loading highly in each of the four factors.

	Factor 1	Factor 2	Factor 3	Factor 4
Number of sows	**0.76**	0.10	0.00	0.02
Number of weaner pen places	**0.56**	0.11	-0.22	0.14
Farrow-to-finisher production[a]	**-0.48**	-0.04	-0.03	0.14
Number of employees	**0.64**	0.13	0.00	0.07
Foreign employees	**0.43**	-0.01	0.15	0.05
Age of newest farm building (in years)	**-0.41**	-0.05	0.12	0.21
Antimicrobial prescription for sows and piglets[b]	**0.35**	0.00	0.22	0.12
Antimicrobial prescription for weaners[b,c]	**0.27**	0.06	0.06	0.23
Number of weaned pigs/sow/year	0.33	0.01	0.03	-0.09
Written biosecurity procedures for employees	0.12	**0.50**	0.18	0.15
Informing employees not to bring food from foreign countries	0.06	**0.45**	0.12	0.28
Informing employees not to feed pigs food leftovers	-0.05	**0.40**	0.01	0.18
Registration of visitors	0.01	**0.30**	0.04	-0.15
Specific pass-through for materials entering the stables[d]	0.07	**0.54**	-0.15	-0.02
Measures taken before materials enter the stables[e]	0.01	**0.50**	0.23	-0.15
Handling diseased pigs after healthy ones	-0.02	**0.42**	-0.07	-0.05
Cleaning and disinfection of equipment before/after use	0.03	**0.32**	-0.08	-0.04
Washing hands between compartments[f]	0.16	**0.30**	-0.14	0.02
Following a strict cleaning procedure of stables	0.17	**0.40**	-0.01	-0.17
Cleaning/changing boots between sections	0.11	**0.38**	0.11	-0.02
Controlling that employees do not bring food from foreign countries	-0.04	0.27	**0.57**	0.29
Controlling that employees do not feed pigs food leftovers	0.06	0.12	**0.63**	0.14
Having problems with rodents	0.07	-0.13	**0.40**	-0.03
Cross-fostering occurring more than once per piglet	-0.19	-0.03	**0.30**	-0.19
Cross-fostering taking place later than 4 days after farrowing	0.14	-0.12	**0.30**	-0.06
Empty transport vehicle picking up sows for slaughter	0.11	0.04	**0.31**	0.01
Exporting weaners	0.15	0.01	**-0.35**	-0.01
Enrolled in the SPF system	0.21	0.18	0.07	**-0.37**
Vaccinating against *Mycoplasma hyopneumoniae*	-0.05	0.06	-0.08	**0.49**
Vaccinating against Porcine Reproductive and Respiratory Syndrome virus	-0.08	-0.10	0.11	**0.43**
Vaccinating against Porcine Circovirus Type 2	0.09	-0.02	-0.02	**0.32**
Vaccinating against *Actinobacillus pleuropneumoniae*	-0.01	0.08	-0.17	**0.30**
Boards used for driving pigs are cleaned regularly	0.09	0.15	-0.28	**-0.34**
Transport vehicle backing up to the stable	0.02	-0.05	0.08	**0.33**

[a]Representing the variable TYPE - assessed by the relationship between number of sows and number of weaner and finisher pen places in each herd
[b]Measured in ADD/100 animals/day
[c]Variable included although the effect was borderline, because variable was not already represented with a higher score in one of the other factors
[d]Foreign materials entering the farm via a specific pass-through (e.g. a UV-cabinet), not through the hygiene lock
[e]Cleaning and disinfection or quarantine period
[f]Between different animal groups or different units (e.g. between farrowing and nursery unit)

The factor plots showed the distribution and variation of herds on two factors at a time. Most of the herds were located close to the centre of the plots meaning there was low variation, except for a few herds. These were the herds scoring high on Factor 1, herds scoring low on Factor 2 and herds scoring high on Factor 4.

As shown by the overlapping ellipses in Fig. 1, SPF herds did not differ much from the non-SPF herds on Factor 2, which was the biosecurity scale. This is probably because the non-SPF herds tended to follow a large proportion of the SPF rules regarding biosecurity. On Factor 1, SPF herds scored slightly higher than non-SPF herds, which indicates that SPF-herds tend to be larger and newer but also had higher productivity and AM-use than non-SPF herds.

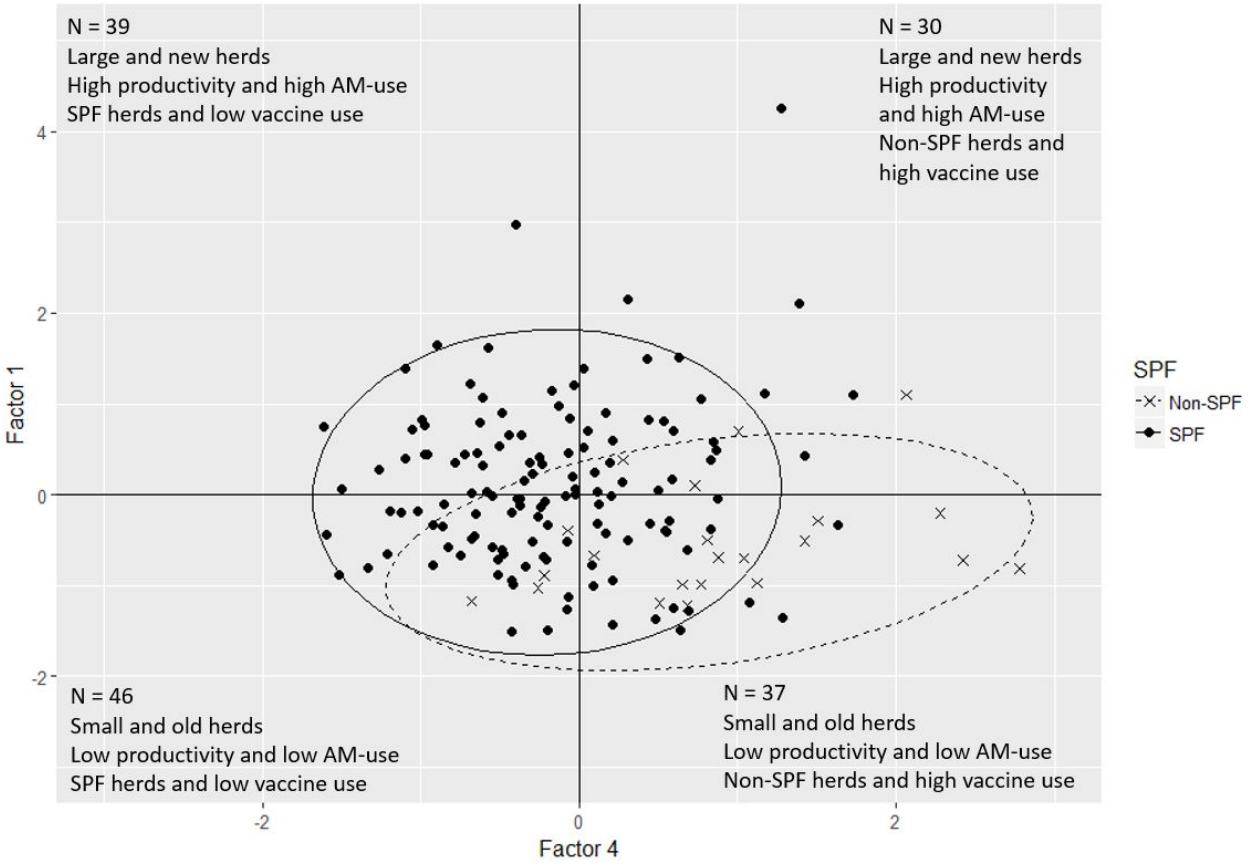

Fig. 3 Factor plot for Factor 1 vs. Factor 4 from the final factor analysis performed on data from 2014 and 2015 representing 152 Danish sow herds. Each point represents an individual herd located according to its scores on the two factors. In each of the four quadrants, the number of observations and an interpretation of the factors scores are given. SPF herds are marked with black dots, and the ellipse with bold line covers 95% of the observations in this group. Non-SPF herds are marked with crosses, and the ellipse with dotted line covers 95% of the observations in this group.

DISCUSSION

The characteristics of the herds included in the study are representative of the Danish sow population. In Denmark, 70% of the sow herds are enrolled in SPF, and for the study population it was slightly higher, with 85% of the herds enrolled in SPF (SPF-Danmark, 2018). The mean number of sows was somewhat lower with 559 sows in the study population and 701 in all sow herds included in the yearly statistics in 2014. The mean herd productivity, measured in

WEANERS PER SOW was on average 30.8. This is very close to the overall mean of 31.1 WEANERS PER SOW in 2014 (SEGES, 2015). The AM prescription level was relatively low, with only a few herds being below the limits of the Yellow Card Scheme (which was 4.3 ADD/100 animals/day for sows including piglets and 22.9 ADD/100 animals/day for weaners in 2014) (Ministry of Environment and Food of Denmark, 2018).

There may be several explanations for the interpretation of the individual factors, which are discussed in the following sections. Thereafter, characterisations of identified herd typologies and recommendations regarding how to improve biosecurity and reduce AM-use while maintaining productivity are given.

Factor 1: Type of herd, productivity and antimicrobial use

With new and modern facilities, it is easier to implement good management practices that prevent disease and affect the productivity positively. New herds are often larger herds, which is why also herd size is included in Factor 1. Productivity correlated with AM-use. Higher AM-prescription levels could be an indication of diseased animals that need treatment resulting in reduced productivity. However, the level of AM-use in Danish pig herds is generally low. Therefore, it is equally likely that herds using more AMs (but still below the Yellow Card Scheme limits) are herds with managers with lower treatment thresholds, maybe due to better education of employees (Backhans et al., 2016). In these herds, the farmer probably reacts quicker and more often to signs of disease, or maybe uses oral group treatment instead of individual injections to prevent infections from spreading. This type of strategy together with the new and modern facilities could explain why these herds had higher productivity.

Factor 2: General biosecurity

Several specific internal and external biosecurity measures grouped into Factor 2, meaning that these biosecurity measures correlated with each other. This suggests that when a farmer has a focus on biosecurity, it is likely that he will have focus on all aspects, not either internal or external biosecurity. Postma et al. (2016a) also found a positive correlation between internal and external biosecurity in a previous study.

Factor 3: Foreign employees, cross-fostering and export

The overall interpretation of Factor 3 was not as easy as for the other three factors. The highest scoring variables covered focus on foreign employees. The other variables had small loadings, and these were related to either internal biosecurity (cross-fostering) or external biosecurity (transportation, problems with rodents and export). Since Factor 2 is already covering biosecurity, Factor 3 is not discussed further in this paper.

Factor 4: Herd health status and vaccine use

Farmers in the Danish SPF system are required to be tested regularly to document freedom from specific pathogens including MYC, APP, PRRS, *Brachyspira hyodysenteriae, Pasteurella multocida, Sarcoptes Scabiei var. Suis* and *Haematopinus suis*. Therefore, if more of these pathogens are present in the herds, it does not make sense for the farmer to pay for enrolment in the SPF system. Conversely, herds free from these infections continue to be part of the SPF system. This could explain why the SPF-enrolment correlated negatively with the use of vaccines in Factor 4. If a farmer uses a vaccine, it is most likely because the herd is test-positive for the specific disease and possibly is experiencing problems that the vaccine can

control (Temtem et al., 2016, Kruse et al., 2017). In this situation, the SPF-enrolment can be considered an indication of health status.

Characterisation of herds and recommendations based on factor plots

Factor 1 vs. Factor 2: One would expect that new herds also had higher standards for biosecurity as seen for the herds in upper right quadrant of the plot. However, this is apparently not the case for the herds placed in the upper left quadrant. These herds probably have the possibility to improve biosecurity. But having high standards for biosecurity is not only a matter of housing conditions, but is also affected by a belief in the cost-effectiveness of biosecurity (Casal et al., 2007). If a farmer does not experience any disease problems, it is likely that he will not implement preventive measures that will cost him extra working hours. However, the herds with lower biosecurity may be at higher risk of introduction of different infections, as well as subsequent spread within the herd, which potentially could have a negative impact on the productivity of these herds in the future. Moreover, if the infection introduced is an exotic virus such as African swine fever or PED-virus, the effect on the entire country may be detrimental. Herds in Denmark are gradually becoming larger. Therefore, for the future of the Danish swine industry, efforts should be made to improve biosecurity in the herds scoring lowest on biosecurity, and among these, the focus should be on the larger herds. This view is shared by the Danish Veterinary and Food Administration, who since autumn 2017, has required veterinarians performing herd health advisory service to advise about biosecurity, once a year in each herd as a minimum (Ministry of Environment and Food of Denmark, 2017). Hopefully, this could improve focus on biosecurity and reduce the risk of introduction of ASF and other exotic diseases.

Opposite to newer herds, older herds do not always have the conditions in farm buildings allowing implementation of high biosecurity. The group of older herds placed in the lower right quadrant of the plot had high biosecurity, but also low productivity. This could consist of herds that earned enough money to run a smaller and well-established production with high focus on biosecurity. Even though they have a low productivity, they may produce more robust weaners compared to large herds with a higher number of WEANERS PER SOW. These herds have low AM-use and high biosecurity, which are characteristics desired by the Danish authorities. The low AM-use could be because of focus on biosecurity to prevent disease problems, a correlation also found in a previous study (Laanen et al., 2013). In addition, it may be hypothesized that it is difficult to become a herd with low AM-use when having high focus on productivity in terms of WEANERS PER SOW. When smaller and younger piglets are weaned they are at higher risk of getting diarrhoea, and therefore in need of treatment (Heo et al., 2013). In a study by Postma et al., (2016b) a weak association was also found between younger weaning age and higher antimicrobial use in farrow-to-finisher pig herds from four EU-countries. Consequently, in the future pig production where use of zinc oxide is not allowed, it may be important to consider productivity differently than today, including how it is possible to run a profitable production with more focus on the weight and age of the weaned piglets (or litter) instead of the number of WEANERS PER SOW.

Factor 1 vs. Factor 4: Herds in the lower right quadrant of the plot used many vaccines, but had a low productivity. A low productivity in these herds is most likely because of disease problems that are not yet under control. The opposite may be applicable for herds in the upper right quadrant of the plot; these herds used vaccines and had a high productivity. It indicates that they vaccinated against diseases present in the herd, but had these under control by using vaccines and probably also other measures. Herds in the upper left quadrant had no or little vaccine use and high productivity, probably because they did not have disease problems

affecting the performance, whereas herds without use of vaccines and low productivity in the lower left quadrant of the plot may be able to increase productivity by starting up targeted vaccination. However, vaccination is a costly measure to implement, and considerations about cost-effectiveness should always be considered before recommending it as a strategy to reduced AM-use or increase productivity. In two studies, by Temtem et al. (2016) and Postma et al. (2016b), herds using vaccines were herds with a higher AM-use, as also seen for some of the herds in the present study. The study by Kruse et al. (2017) showed that initiation of vaccination was not generally associated with a positive or negative change in AM-use over time. However, herds with high AM-use succeeded in reducing the AM-use over time, some using vaccines as a strategy, while others (including the non-vaccinating herds) probably used other measures. Another way to cope with disease problems in a herd could be to eradicate the infection. In Denmark, it is difficult to use medical eradication, because of the Yellow Card Scheme limits. There are other ways to eradicate infections, but they are either costly (e.g. stamping-out of the disease) or associated with a probability of failure leading to potential re-infection (e.g. the eradication of MYC in Maes et al. 2008).

Again, herds in Denmark are becoming larger over time, but it may be important to bear in mind that most of the herds are now located in the lower left quadrant of the plot. These herds had a low AM-use and high health status, and therefore, low vaccine use. However, the productivity was low. Therefore, when increasing in herd size to become more effective it is important to consider the importance of a high number of WEANERS PER SOW and how it could affect the AM-use as already discussed.

This study shows that it is important to have country-specific studies when revealing joint effects between data on AM-use, biosecurity, productivity and use of vaccines, since local conditions including restrictions from authorities, traditional farming practices and current national conditions play a strong role. This study found a positive correlation between AM-use and productivity. Productivity did not seem to correlate with biosecurity or vaccination. Therefore, it is important to consider alternative ways of keeping up a sustainable production in future pig production without the use of zinc oxide and continued focus on reducing AM-use.

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CHRONIC DISEASES

FURTHERING OUR UNDERSTANDING OF THE PERSISTENCE OF *FUSOBACTERIUM NECROPHORUM* IN SHEEP: A LONGITUDINAL FIELD-BASED STUDY

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SUMMARY

Ovine footrot is the most common cause of lameness on UK sheep farms. *Fusobacterium necrophorum* is a secondary pathogen that increases footrot severity. The primary reservoirs for *F. necrophorum* in sheep were believed to be sheep faeces and the environment, however, no studies had demonstrated the presence of *F. necrophorum* at either of these sites. A longitudinal study was conducted to determine reservoir sites of *F. necrophorum* in ovine footrot. Quantitative PCR (qPCR) was used to detect and quantify *F. necrophorum* in foot swabs, mouth swabs, faeces, soil and grass. Multiple locus variable number tandem repeat analysis (MLVA) community typing was used to investigate variation in *F. necrophorum* between samples. Contrary to prior assumption, the environment was not a significant reservoir of *F. necrophorum*. *F. necrophorum* persisted in sheep, primarily on feet with footrot. Mouths and faeces were an intermittent reservoir for the strains of *F. necrophorum* involved in footrot.

INTRODUCTION

Footrot is an infectious dermatitis of the interdigital skin of sheep that causes lameness. This leads to poor welfare (Ley et al., 1995; Goddard et al., 2006), poor health and reduced productivity (Marshall et al., 1991; Nieuwhof et al., 2008; Wassink et al., 2010), with resulting economic losses for sheep farmers. Footrot is reported in sheep farming countries worldwide, and in the United Kingdom (UK) it is the most common cause of lameness in sheep (Grogono-Thomas & Johnston, 1997; Kaler & Green, 2008; Winter et al., 2015).

Fusobacterium necrophorum is a Gram-negative, rod-shaped anaerobe. In footrot it is a secondary pathogen that increases disease severity (Beveridge, 1941; Roberts & Egerton, 1969; Witcomb et al., 2014). Current understanding of the reservoirs of *F. necrophorum* in sheep is poor. The literature states that the environment and sheep faeces are the primary reservoirs for *F. necrophorum* in footrot (Roberts & Egerton, 1969; Langworth, 1977), however, in a recent study *F. necrophorum* was not detected in soil from sheep pasture or sheep faeces (Witcomb, 2012). *F. necrophorum* can be detected on the healthy feet and in the mouths of sheep (McCourtie et al., 1990; Witcomb, 2012; Witcomb et al., 2014; Frosth et al., 2015; Maboni et

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al., 2016), but these data are mainly cross sectional. There have been no longitudinal studies on persistence of *F. necrophorum* at any of these sites under natural conditions.

The role of the environment in transmission of *F. necrophorum* has been observed in deer and cattle (Monrad et al., 1983; Edwards et al., 2001), but only under conditions of high stocking density and high rainfall. Contamination of the feet of sheep with faeces results in colonisation with *F. necrophorum* (Roberts & Egerton, 1969), but again this was demonstrated in wet conditions and at high stocking density. There are no observational studies that link *F. necrophorum* presence at reservoir sites to footrot in sheep kept at pasture.

The aim of this study was to determine sites of persistence of *F. necrophorum* in sheep and their environment using longitudinal data on *F. necrophorum* presence and load in samples collected from feet, mouths, and faeces of sheep, as well as soil and grass from sheep pasture. Footrot lesions in sheep were also recorded to understand associations between changes in presence and load of *F. necrophorum* at reservoir sites and footrot in the flock. Additionally, the aim was to use multiple locus variable number tandem repeat analysis (MLVA) for these samples to better understand variation in *F. necrophorum* between sites and over time.

MATERIALS AND METHODS

Study design and sampling procedures

A longitudinal study of 40 sheep and their pasture was conducted for 20 weeks from February to July 2015. The study was approved by the University of Warwick's local ethics committee (AWERB.33/13-14). Faecal sampling of the sheep was carried out under a UK Home Office Licence (PPL 70/8392).

The study field was sampled on 7th February 2015 when the previous group of sheep were moved off the field. Soil and grass samples were collected from near a ring feeder and water trough in the field because these were expected to be high traffic areas where sheep frequently collected, and one low traffic area (20m × 20m area in centre of field). The pasture was left un-grazed for 10 days and sampled again on 17th February 2015.

On 18th February 2015, 120 ewe lambs were observed for lameness and divided into lame and non-lame groups. All four feet of the non-lame ewe lambs were examined and scored for lesions of interdigital dermatitis (ID) and severe footrot (SFR) as described by Moore et al. (2005) (Table 1).

Swab samples (EUROTUBO Collection swab; Delta Lab, Rubi, Spain) were collected from the interdigital skin of each foot, and the gingival crevice of the front lower incisors. A rectal faecal sample was collected from each of the sheep, or a rectal swab was taken if insufficient faecal material was present. Forty healthy (ID ≤ 1, SFR = 0) ewe lambs were selected as the study sample and moved onto the study pasture. The study sample and pasture were examined and sampled every week from 18th February 2015 to 1st July 2015. There were a total of 20 sampling occasions, termed week 1 - week 20. A summary of the samples collected is presented in Table 2.

Table 1. Scoring system for lesions of interdigital dermatitis (ID) and severe footrot (SFR) from Moore et al. 2005.

Lesion score	Description
Interdigital dermatitis	
0	Clean interdigital foot with no dermatitis (scald) lesion or fetid smell
1	Slight interdigital dermatitis, irritation of the skin but dry
2	Slight interdigital dermatitis with a fetid smell, < 5% skin affected
3	Moderate interdigital dermatitis with a fetid smell, 5-25% skin affected
4	Severe interdigital dermatitis with a fetid smell, > 25% skin affected
Severe footrot	
0	A clean digit with no lesion
1	An active or healing footrot lesion with a degree of separation of the sole
2	An active footrot lesion with a marked degree of separation of the sole
3	An active footrot lesion with extensive under-running of the wall hoof horn (may include under-running of the sole)
4	An active footrot lesion with complete under-running of the wall hoof horn (may include under-running of the sole)

Table 2. Samples collected during the longitudinal study by visit and in total.

Sample type		Number collected per visit	Total number collected
Soil	*High traffic*	12	252
	Low traffic	10	210
Grass	*High traffic*	0-6[a]	73
	Low traffic	5	105
Foot swab		160	3192[b]
Mouth swab		40	798[b]
Faeces		40	798[b]

[a]Grass collected when present in high traffic areas; this varied by week
[b]One sheep missed two sampling points

DNA extraction

DNA was extracted from all environmental samples (soil and grass) plus a subset of sheep samples. This included samples from 19 diseased sheep from 2 weeks before a period of footrot (or start of the study) to 2 weeks after the period of footrot (or end of the study). Samples from every fourth week from two sheep that scored ID0 and SFR0 for the duration of the study were analysed. Samples were analysed from weeks 1-3 for these 21 sheep, plus a further randomly selected nine sheep. DNA was extracted from all sample types using the method described by Purdy (2005). DNA from soil, grass and faecal samples was further purified using polyethylene glycol (PEG) precipitation based on a method from Selenska and Klingmüller (1991).

Quantitative PCR (qPCR) for *F. necrophorum*

A qPCR targeting the *rpoB* gene of *F. necrophorum* was used to analyse extracted DNA as described by Witcomb et al. (2014). qPCR reactions were carried out in a final volume of 25µl using 2 × TaqMan® Universal Mastermix (Applied Biosystems, Warrington, UK). Samples were initially screened as a singlet, and those that were positive for *rpoB* were then analysed

in triplicate. Only samples with all three replicates positive were counted as positive results, and the mean number of *rpoB* copies μl^{-1} was calculated from the three replicates. The number of *rpoB* copies per swab, or per gram of soil, grass, or faeces was calculated.

Multiple locus variable number tandem repeat analysis (MLVA) of *F. necrophorum* positive samples

Foot swabs, mouth swabs and faecal samples that were positive for *F. necrophorum* by qPCR were analysed using the MLVA community typing scheme described by Clifton et al. (2018).

Statistical analysis

All statistical analyses were carried out using the R (v3.3.2) statistical environment (R Development Core Team, 2008) with the R studio user interface (v1.0.136). (Bacterial load data + 1) were positively skewed and so $\log_{10}$ transformed for statistical analyses. Footrot was defined as a foot scoring ID > 1 or SFR > 0.

Two-level binomial mixed effects models were used to determine associations between footrot status and load of *F. necrophorum* from positive foot swabs, mouth swabs, and faecal samples. The models took the form shown in Eq. (1).

$$Logit(\pi_{ij}) = \beta_0 + \beta x_i + u_j \quad (1)$$

Logit(π_{ij}) is the log odds of the probability that footrot was present, β_0 is the constant, βx is the fixed effect ($\log_{10}$ transformed load of *F. necrophorum*) which varies at *i* (observation), with residual variance estimates (u_j) at foot (foot swabs) or sheep (mouth swabs and faecal samples). Models were constructed using the lme4 package in R (Bates et al., 2015). Associations between load and footrot status were considered significant when 95% confidence intervals of the coefficient for load did not include 0.

Non-parametric maximum likelihood estimation (Kaplan-Meier estimate) of survival of *F. necrophorum* positive foot swabs was carried out. The event (failure) was a foot becoming negative for *F. necrophorum*. Data were interval censored: the time period was grouped into weekly intervals, with events occurring during these intervals but exact time of events being unknown. The Wilcoxon two sample permutation was used to test for differences in survival probabilities between healthy feet and feet with footrot. Survival analysis was carried out using the interval package in R (Fay & Shaw, 2010).

RESULTS

Detection of *F. necrophorum* in pasture samples

There were 4/462 (0.9%) soil samples and 0/178 (0%) grass samples positive for *F. necrophorum*. Three of four positive soil samples were from the baseline samples taken 10 days before the sheep moved onto the study pasture. The fourth positive sample was from the second visit. All positive soil samples were from a high traffic area (ring feeder).

Detection and load of *F. necrophorum* in sheep samples

The numbers of positive foot swabs, mouth swabs and faecal samples are shown in Table 3. *F. necrophorum* was detected in sheep samples at all time points except weeks 18 and 20. Foot swabs were the only samples where *F. necrophorum* was detected after week 10, with the exception of one positive mouth swab in week 17.

Table 3. Frequency of detection and load of *F. necrophorum* in sheep samples.

Sample type	Frequency of detection		*rpoB* copies in positive samples	
	Number	Percentage	Minimum	Maximum
Foot swabs	85/1106	7.7	1.03×10^2 swab^{-1}	8.50×10^7 swab^{-1}
Mouth swabs	21/284	7.4	1.82×10^2 swab^{-1}	1.67×10^6 swab^{-1}
Faeces	11/283	3.9	2.18×10^5 g^{-1} [a]	1.89×10^7 g^{-1}

[a]There was one rectal swab positive for *F. necrophorum* which had a load of 2.12×10^3 *rpoB* copies swab^{-1}

Detection and load of *F. necrophorum* on foot swabs

Feet with footrot were more likely to have higher $\log_{10}$ (load of *F. necrophorum)* than healthy feet (OR 2.12, 95% CI 1.36-3.61). *F. necrophorum* was detected on the same foot for between 1 and 12 consecutive weeks, and survival probability was greater on feet that had footrot than those that were healthy ($p < 0.01$; Fig. 1).

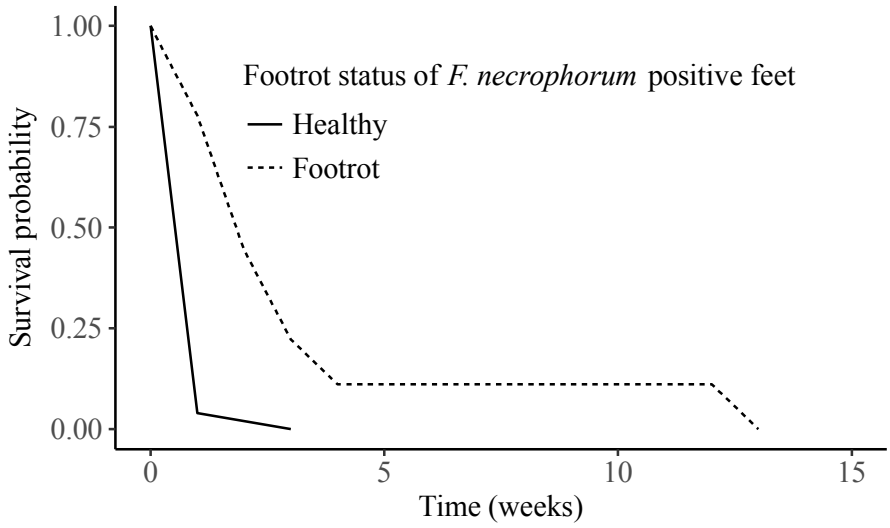

Fig. 1 Survival probability of *F. necrophorum* on feet. The probability of feet positive for *F. necrophorum* remaining positive over time is plotted for feet that had footrot whilst positive and those that were healthy whilst positive.

Detection and load of *F. necrophorum* on mouth swabs and in faecal samples

Load of *F. necrophorum* on mouth swabs and in faecal samples was not associated with footrot status. Eight sheep had mouth swabs positive for *F. necrophorum* (Fig. 2). Fifteen of twenty-one (71.4%) positive mouth swabs came from three sheep that had multiple positive mouth swabs (sheep 03520, 03463 and 03539; Fig. 2). Ten of the eleven positive faecal samples were from two of these sheep (sheep 03520 and 03463; Fig. 2). The longest period of

consecutive detection of *F. necrophorum* from mouth swabs was 6 weeks (sheep 03539; Fig. 2), and in faecal samples was 4 weeks (sheep 03520).

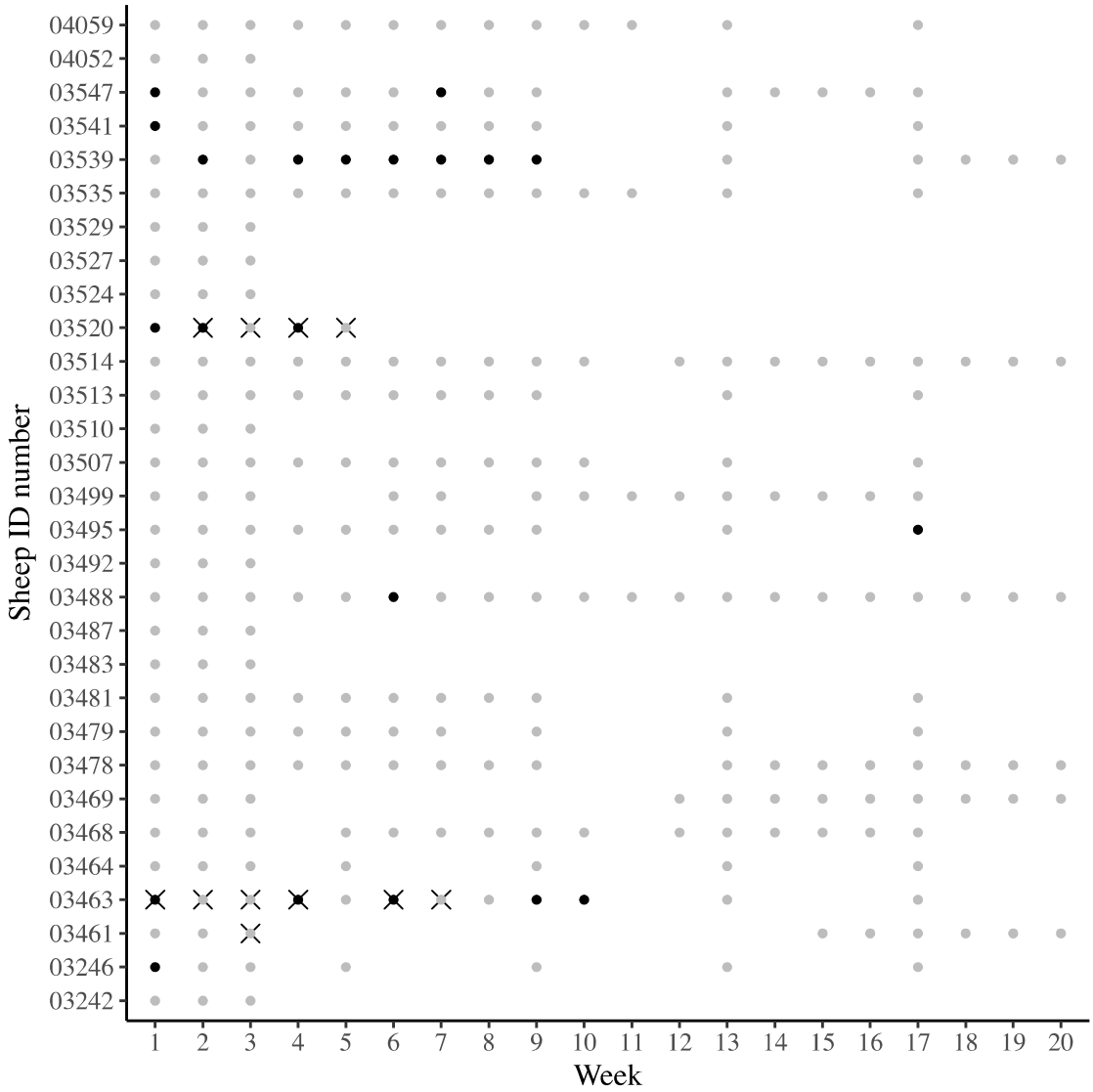

Fig. 2 Detection of *F. necrophorum* in mouth swabs and faecal samples by sheep. Grey circles show samples analysed, black circles show positive mouth swabs and black crosses show positive faecal samples.

MLVA of sheep samples

There were 109 sheep samples analysed by MLVA, and a full (three loci) or partial (one or two loci) profile was obtained from 68 (62%) of these samples. A full profile was obtained from 24/79 (30%) foot swabs, 4/19 (21%) mouth swabs and 2/11 (18%) faecal samples. Partial profiles were generated from 16 foot swabs, 13 mouth swabs and nine faecal samples.

At loci Fn13 and Fn42, one variant dominated per locus and was present in 31/33 (94%) and 54/57 (95%) profiles respectively (Table 4). There was a greater degree of variation at locus Fn69, with the most dominant variant (Fn69.3) accounting for 33/60 (55%) of variant detections (Table 4). Variants Fn69.3 and Fn69.4 were detected on feet. These variants

accounted for 1/13 (8%) and 2/13 (15%) mouth swab detections, and 6/11 (55%) and 0/11 faecal sample detections respectively.

Table 4. Frequency of detection of MLVA variants in sheep samples.

MLVA variant	Foot swabs (healthy) n=23	Foot swabs (footrot) n=16	Mouth swabs n=17	Faecal samples n=11	Total n=68
Locus Fn13					
13.1a	1	0	1	0	2
13.2	8	15	5	3	31
Locus Fn42					
42.4	1	0	0	0	1
42.5	16	16	13	9	54
42.6	0	0	1	0	1
42.7	1	0	0	0	1
Locus Fn69					
69.1	0	0	1	0	1
69.2	0	0	9	5	14
69.3	11	15	1	6	33
69.4	10	0	2	0	12

DISCUSSION

F. necrophorum was detected at low frequency in soil and was not detected on grass. Detection of *F. necrophorum* in soil occurred transiently in one high traffic area, suggesting contamination of the environment by sheep. Outbreaks of necrobacillosis in other ungulates are reported in connection with animals gathering at feed or watering stations during periods of high rainfall (Monrad et al., 1983; Edwards et al., 2001; Handeland et al., 2010). The evidence from the current study suggests that soil is not a normal site for *F. necrophorum* persistence, and that transmission via the environment is generally low. This is a complete paradigm change from the previous assumption that *F. necrophorum* is ubiquitous in the environment of sheep, and that the environment represents a significant reservoir for footrot.

Feet were the only site where *F. necrophorum* was consistently detected over the entire study period, suggesting that feet were the primary site for persistence of *F. necrophorum* within this flock. *F. necrophorum* was more likely to persist on feet with footrot than healthy feet, and the majority of healthy feet were only positive for 1 week. This suggests that although *F. necrophorum* can be detected on healthy feet, they are only transiently positive and therefore unlikely to represent a significant site of persistence. Increased loads of *F. necrophorum* were found on feet with footrot, and feet with footrot may therefore play a significant role in transmission of *F. necrophorum* within the flock. However, there is recent evidence to suggest that shedding high loads of bacteria does not necessarily have a proportionate effect on transmission (Spencer et al., 2015), and therefore the role of footrot in *F. necrophorum* transmission requires further investigation.

The current study provides the first direct evidence that *F. necrophorum* can be shed in sheep faeces, but suggests that shedding is not widespread amongst sheep. In two of the three sheep that shed *F. necrophorum* in faeces, the length of the shedding period could not be fully

determined because shedding was still occurring at the last sample analysed. It is possible that shedding is a transient property, as suggested by Spencer et al. (2015), that could occur in any individual. Alternatively, it may be specific to certain individuals based on *F. necrophorum* being a stable member of the gastrointestinal microbiota in these sheep and not others.

This study provided evidence for variation in *F. necrophorum* within and between sites in sheep (feet, mouths and faeces). The Fn69.3 and Fn69.4 MLVA variants detected on feet were intermittently present in mouths and faeces, suggesting that these sites could be transient reservoir sites of *F. necrophorum* in footrot. It is possible that these sites could be relevant for *F. necrophorum* persistence within a flock in the absence of footrot lesions, but further investigation would be necessary to confirm this. The presence of additional *F. necrophorum* variants in mouths and faeces suggests that there may be strains that do not possess the characteristics to be able to survive and persist on feet. If this suggestion was confirmed, it would significantly improve current understanding of reservoirs of *F. necrophorum* in footrot because only sites containing the strains relevant for footrot would need to be considered as potential reservoirs.

The findings from the current study highlight variation in *F. necrophorum* between feet, mouths and faeces, however, they are based on variation at one MLVA locus, Fn69. There is almost certainly more variation in *F. necrophorum* than detected from this single locus. Future work using genome sequencing of *F. necrophorum* isolates from different sites would increase understanding of the strains of *F. necrophorum,* their habitat(s), and behaviour over time.

The results of this study indicate that contrary to existing assumptions, *F. necrophorum* is not frequently present on sheep pasture. In contrast, sheep are the main reservoir of *F. necrophorum.* The results suggest that footrot facilitates persistence of *F. necrophorum* on feet, and that mouths and faeces of sheep may intermittently harbour strains of *F. necrophorum* involved in footrot.

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ASSOCIATION BETWEEN DAM STATUS AND OFFSPRING *MYCOBACTERIUM AVIUM* SUBSPECIES PARATUBERCULOSIS INFECTION IN A LONG-TERM LONGITUDINAL STUDY

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SUMMARY

A longitudinal study was carried out, following a large cohort of dairy cows in six UK herds, recruited in 2012-2013. Individuals entering the milking herd were routinely monitored for presence of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) using quarterly milk ELISA testing. Using a Cox proportional-hazards regression model the relationship between time until first detection of infection and dam MAP status was investigated, allowing for both management and individual animal risk-factors. The magnitude of the effect of dam status was then compared with that of other risk-factors in order to understand its relative importance. When compared to negative dams, and stratifying by herd, having a positive dam at time of calving more than doubled the hazard of testing positive. Further positive associations were found with dams becoming positive *after* the birth of the subject. Dam status had the largest effect on disease outcome.

INTRODUCTION

Understanding of the epidemiology of Johne's disease is hampered both by poor diagnostic test sensitivities and by the long incubation period, which lead to slow research progress, and notorious difficulties with control (Lombard et al., 2005; Dorshorst et al., 2006; Meyer et al., 2018). The disease itself is caused by the bacterium *Mycobacterium avium* subsp. *paratuberculosis* (MAP), an intracellular organism affecting the lower small intestine (Harris & Barletta, 2001; Whittington, 2010). Within Great Britain, a cross-sectional study has previously estimated the prevalence of MAP-infected herds as ranging between 59% and 77% (Velasova et al., 2017), whilst a separate study in the South West of England has put the number of herds with at least a single seropositive animal as high as 75-78% (Woodbine et al., 2009). Initial MAP infection is believed to be acquired within the first few days of life, but with clinical signs often not appearing until 3-4 years of age (Sweeney, 1996).

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These clinical signs principally present as chronic weight loss and a characteristic diarrhoea (Whitlock & Buergelt, 1996). Diarrhoea may be intermittent, coming in waves, and may be accompanied by a "bottle jaw" appearance as fluid accumulates beneath the jaw due to protein loss (Sweeney, 2011). Such animals continue to deteriorate and will usually be culled on welfare grounds.

Transmission of MAP to calves is through ingestion of the bacteria, either through the oro-faecal route, or through drinking contaminated milk (Whittington & Sergeant, 2001; Slana et al., 2008). There are no clinical signs at this stage of infection. Bacteria reside within intestinal cells invoking immune responses within the gut. Over time this leads to thickening of the gut lining, with consequent reductions in intestinal ability to absorb nutrients, leading to the clinical signs typically associated with the disease (Tiwari et al., 2006). During the early stages of disease development, infection cannot be detected by either faecal or serological testing. As disease develops, shedding may begin, typically in older youngstock or adult cattle (Nielsen & Ersbøll, 2006; Mitchell et al., 2011). These animals represent an important source of infection to the herd as there may be a large number of such animals, and yet clinical signs are unapparent. From the onset of clinical signs, individuals are likely to be shedding high burdens of MAP in faeces, colostrum, and milk, typically in an intermittent fashion (Whittington & Sergeant, 2001). Clinical signs and high shedding episodes will often be associated with stressful events such as calving (Martcheva et al., 2015).

Economic losses due to Johne's disease are difficult to determine, but are associated with both increased culling costs/mortality, and subclinical costs including weight loss, reduced milk yield and poor fertility (Smith et al., 2009). Further losses may consequently be caused by treatment failures and the implementation of diagnostic tests, as well as the establishment of new management strategies targeted at disease control. Failure to truly understand prevalence rates within infected herds makes estimates of true financial losses likely to be underestimates.

Treatment for Johne's disease is not a viable option, and so herd-level control strategies are based upon prevention of transmission and removal of infectious individuals. Test strategies are now widely adopted in the UK to address these needs (Geraghty et al., 2014). This approach is based upon the milk ELISA which can be applied to milk routinely collected as part of individual cow screening. Cows are typically sampled on a quarterly basis for Johne's disease. Prevention of transmission focuses on the periparturient period, targeting the relationship between the susceptible, new-born calf, and adult animals within the herd. Different management protocols are recommended to reduce new cases of Johne's disease within the herd, but detailed information on the relative importance of individual routes of infection are unknown (Geraghty et al., 2014; Garcia & Shalloo, 2015). In practice, known MAP-positive individuals showing no clinical signs are generally retained within the milking herd whilst they remain financially viable, in order to reduce the number of culls carried out. Cows known to be infected will be served to beef bulls, and their offspring reared separately from the milking herd for meat production. However, a significant number of replacement dairy heifers are born to MAP-infected dams, either because they were born prior to detection of MAP, or due to an existing pregnancy at the time of the diagnosis.

This study sets out to investigate the relationship between the dam's MAP status and the likelihood of infection in her offspring. A cohort study was carried out, recruiting calves at birth from known Johne's-infected herds investigating calves born prior to, and after, the detection of MAP in the dam. The results of this study will be of interest to both farmers and production animal veterinarians, to guide their approach to disease management.

MATERIALS AND METHODS

Study herds and animals

Nearly 600 heifer calves were recruited in six herds at the point of calving during 2012 and 2013. Two herds were managed separately on the same holding, so there were five different farms included, represented by six herds (the herds are referred to as A-F). A schematic of the study design is presented in Fig. 1. Observations included in the analysis were: the dam's future and current Johne's disease status at the time of calving; the ease of calving; the source, quantity, and method of delivery of colostrum; the cleanliness of the calving yard and the number of cows within it; whether the calf suckled the dam or another cow; how soon colostrum was given and of what quality; how long the calf remained in the calving pen after calving; and the size of the calf. Analyses were stratified by herd.

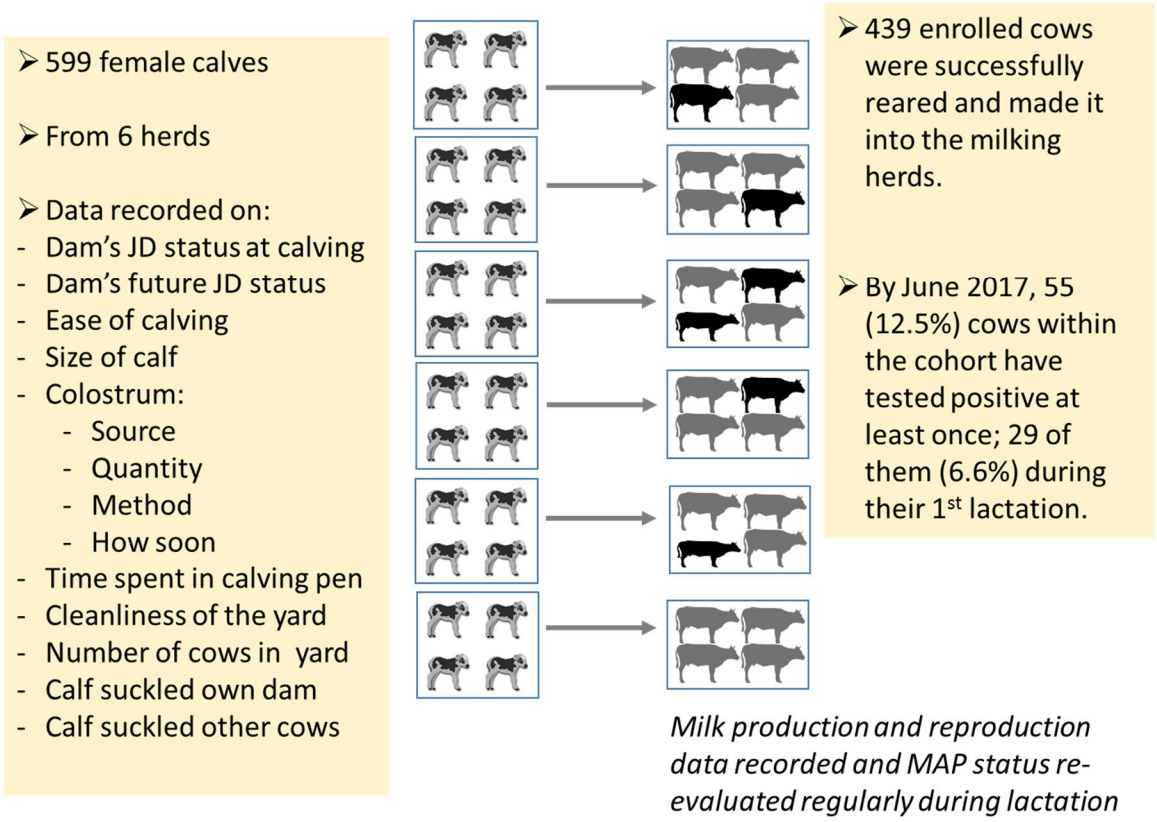

Fig. 1 Schematic of the cohort study for investigation of the relationship between dam and offspring MAP status.

After first calving, all cows within the cohort were subjected to regular individual cow milk ELISA testing, carried out using one of two commercial laboratories, either National Milk Records (NMR) or Cattle Information Service (CIS). For the purposes of this study, results greater than 20% sample to positive ratio (S/P) but below 30% S/P were classified as inconclusive, and results over 30% S/P were considered positive.

Data analysis

The analysis of the dataset was carried out in three sequential steps:

i) Descriptive statistics to summarise MAP status and frequency of exposure variables across herds and to assess whether some factors were too homogeneous within a herd to allow subsequent herd-stratified analyses.

ii) Univariable analyses stratified by herd by means of univariable stratified Cox regression. In this step all variables where considered one at a time.

iii) Multivariable analyses stratified by herd by means of multivariable stratified Cox regression. Variables showing a certain degree of association in the previous step were selected and assessed simultaneously as part of the same regression model.

RESULTS

Over two thirds of enrolled cows were successfully reared and entered the milking herds. By the end of the study period more than 10% of cows within the cohort had tested positive for MAP at least once (Fig. 2). The incidence of new infections in the six herds varied over time and between herds, when taking account of all cows in the herd (both those that formed part of the cohort, and the remaining cows in milk) (Fig. 2).

As expected, the source, quantity, and delivery method for colostrum feeding were found to be highly correlated within a farm (i.e. very small within-herd variability), therefore these factors were dealt with separately.

Analysis of dam status effect

A significant association was found between dam status and an individual becoming test positive for MAP. No other risk factor had a significant association with MAP status and although different models incorporating dam status and co-variates were explored, no model including multiple variables was found to explain the observed MAP seroconversions better than that incorporating only dam status stratified by farm. The proportional hazards assumption was met for this model.

When compared to negative dams, and stratified by herd, having a positive dam at the time of calving more than doubled the hazard of testing positive. Negative dams who seroconverted later in life, produced offspring with hazards close to those of dams already positive when the calf was born. Individuals born to dams that tested positive within the first 12 months of their birth and those whose dams tested positive more than a year later had hazards of testing positive between three and four times greater than calves born to seronegative dams.

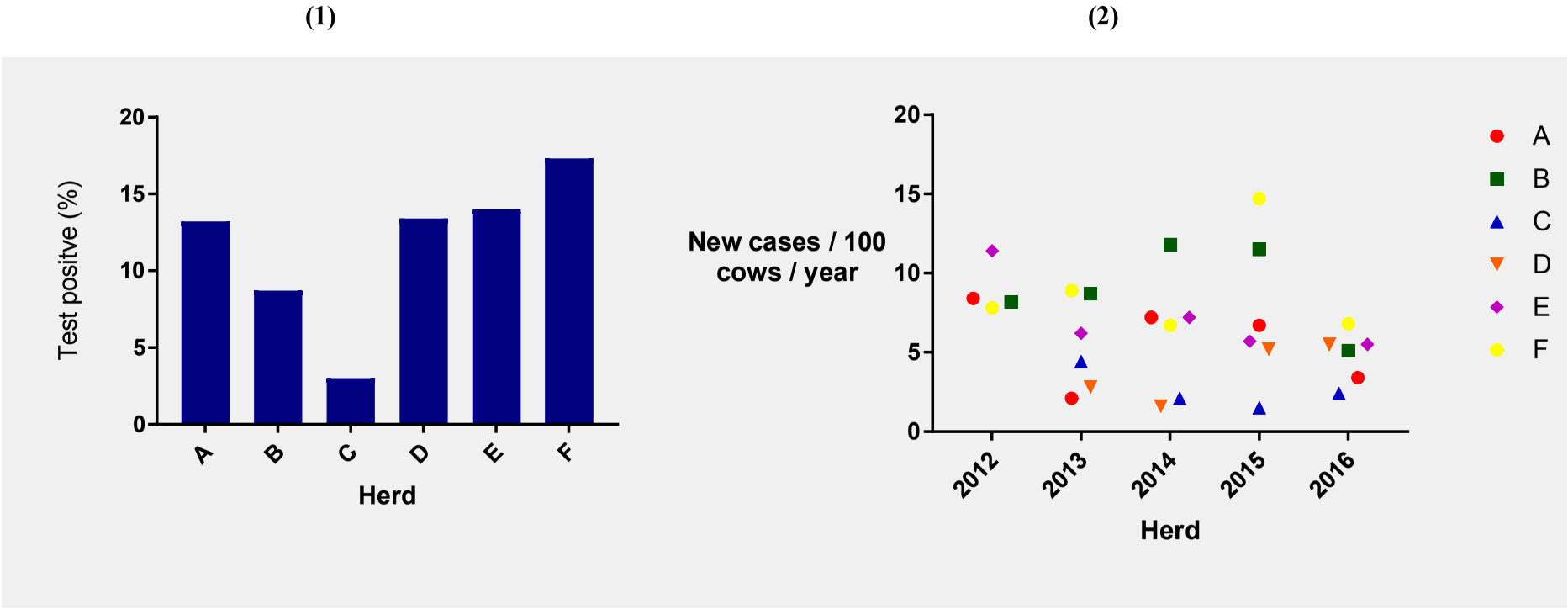

Fig. 2 Proportion of enrolled cows that tested positive for MAP in each herd (1) and incidence of new cases of Johne's disease in each herd from 2012 to 2016 (2).

DISCUSSION

The purpose of this study was to understand whether cows born to MAP-negative dams differed in their likelihood of testing MAP-positive themselves, depending upon whether the dam converted to MAP-positive *after* the birth of the subject. Current understanding of Johne's disease transmission is that calves born to MAP-positive dams are at a higher risk of becoming infected as such dams are expected to be excreting high quantities of MAP in colostrum and faeces which may contaminate the calf during parturition or suckling. However, prior to MAP being detectable in milk ELISAs, levels of shedding are believed to be low enough so as not to pose a significant risk to neonatal calves. This research suggests that this is not the case, and that in fact all offspring of a MAP-positive dam are more likely to become infected than those from a dam which never tests positive.

Whittington & Windsor (2009) state that, on the basis of numerous studies, the relative importance of *in utero* transmission of Johne's disease varies from farm to farm. The issue of *in utero* transmission is commonly not addressed in management policies. The implications of the present study however, do raise the possibility that this may be occurring. Given that dams are likely themselves to have been infected as calves, then once MAP-positive status has been diagnosed, it is highly likely that this animal can be assumed to have been infected through her entire life, including the times of earlier pregnancies. Opportunities for her to have infected an earlier calf therefore include *in utero* transmission of infection, or a temporary immunosuppression in the periparturient period allowing for temporary shedding.

Results from this study appear to be robust, given the study size and the strength of association found. It will be of interest to follow this population as the study subjects are continually monitored. It is unlikely, but possible, that a few subjects may be reclassified as there are a small number of negative dams still in the milking herds who may eventually test MAP-positive. However, these remaining animals are older cows which, if they were ever to seroconvert, would have been expected to have done so by this stage. Long-term datasets such as this one, examining this issue are rare, and so this analysis is expected to have made an important contribution to current understanding.

Stochastic modelling of Johne's disease control using a test and cull strategy, carried out by Lu et al. (2010) showed that many herds would take more than 20 years to eliminate disease, and in a separate study by Ferrouillet et al. (2009) elimination had not been achieved in any of six herds after 5 years of control plan implementation. General agreement is that complete elimination from a herd is a long-term goal at best. Current advice is to minimise contact between calves and adult cattle and to serve MAP-positive cattle to beef semen to avoid the retention of their offspring in the milking herd. This advice does not address the previous progeny of MAP-positive cows and the current study suggests that this may be a serious oversight. The implications about placing management restrictions on all prior offspring of MAP-positive cows may be significant. However, consideration ought to be given to automatically considering such animals as MAP-positive, at least in the later stages of a disease management programme when the numbers of such interventions would be reduced. Leaving these animals within the herd, with no restrictions placed upon them, may be a major contributor to the long duration of elimination programmes.

If management strategies are to adapt to take into account the older offspring of MAP-positive cows, then the use of economic simulations to evaluate the potential benefits would be extremely useful. Such models would need to consider the likelihood of offspring being

infected, and current expected costs of Johne's disease to herds. It seems likely that the severity of interventions warranted may change with the prevalence of disease, with culls perhaps only considered in the very late stages of elimination.

This study has made use of a long-term dataset to investigate the impact of dam status upon the likelihood of offspring becoming MAP-positive. It presents evidence to support the current understanding that MAP-positive dams are more likely to have MAP-positive offspring than MAP-negative dams, but has also additionally shown that offspring are more likely to seroconvert if their dam seroconverts later in life. These findings have interesting management repercussions for dairy farmers, and the economic implications of altered interventions are well worth considering as a result.

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HERD CHARACTERISTICS, WILDLIFE RISK AND BACTERIAL STRAIN GENOTYPES IN PERSISTENT BREAKDOWNS OF BOVINE TUBERCULOSIS IN NORTHERN IRISH CATTLE HERDS

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SUMMARY

Prolonged and recurrent (i.e. "chronic") bovine tuberculosis (bTB) breakdowns are an important issue in Northern Ireland (NI), disproportionally contributing to eradication efforts. To understand the phenomenon better, the risk factors for chronic breakdowns were determined as part of a retrospective observational study. They were found to include herd size, the presence of associated herds, average patch bTB prevalence, and the buying-in of cattle. Critically, chronic breakdown risk was associated with increasing presence of different bTB molecular strains, suggesting that chronic infection arises from introductions of bTB from multiple sources. Additionally, different *M. bovis* molecular strains were themselves associated with different levels of chronic breakdown risk. Whilst the distinctions were subtle, this offers an important opportunity for disease management via early identification of potentially chronic episodes. Wildlife likely contribute towards maintaining local bTB prevalence and via spillback introduction, but may not be critical in driving chronic infection within herds.

INTRODUCTION

Bovine tuberculosis (bTB; caused by *Mycobacterium bovis*) is endemic in Northern Ireland (NI), with a herd-level incidence of 8.23% and rising (DAERA, 2017). The state-led NI bTB eradication programme implemented in NI is compliant with EU Directive 64/432/EEC (as amended), consisting of a test-and-slaughter policy (Abernethy et al., 2006), with routine abattoir surveillance for bTB lesions and follow-up laboratory confirmation. Animals are annually tested using the Single Intradermal Comparative Cervical Tuberculin (SICCT) test. Any herds containing "reactor" animals (i.e. SICCT-positive) are placed under trading restrictions which prohibit movements out of the herd, except in very specific circumstances e.g. to a slaughterhouse. Once a breakdown is confirmed, the herd must pass two full-herd tests, each 60 days apart, resulting in a restriction period of usually 120 days.

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The cost of this eradication programme exceeds £30 million per annum, and the consequential trading restrictions and production losses cause considerable disruption to the farming community (Robinson, 2017). Some 18% of herds which suffer a bTB breakdown experience prolonged episodes lasting over one year, or subsequent recurring episodes in the years following an initial breakdown, i.e. "chronic" breakdowns (AFBI - unpublished data). Given the disproportionate contribution of chronic breakdowns to eradication costs, it might be presumed that the risk factors for chronic breakdowns would be well-understood. However, only one study thus far has specifically investigated the herd-level risk factors associated with chronic breakdowns in NI (Doyle et al., 2016).

Doyle et al. (2016) showed that some of the herd-level risk-factors involved in chronic breakdowns in NI include herd-level risk-factors for bTB in general (Skuce et al., 2012; Broughan et al., 2016). These include herd-size (Brooks-Pollock & Keeling, 2009), herd type (with dairy herds experiencing greater risk) (Lahuerta-Marin et al., 2016), and inward cattle movements (Gilbert et al., 2005). However, a number of key risk-factors were absent from this study including contiguity and proximity to other cattle farms (White et al., 2013), and abundance of the European badger (*Meles meles*) (Griffin et al., 2005; Reilly & Courtenay, 2007; Byrne et al., 2015). The *M. bovis* genetic strain data may also be important, as evidence indicates that different pathogen molecular types exhibit subtle differences in the proportion of animals with visible lesions (Wright et al., 2013a), and in detectability (Wright et al., 2013b).

What follows therefore, is a retrospective observational evaluation of the herd-level risk factors associated with chronic bTB infection in NI, including wildlife data and *M. bovis* genetic strain data, for (i) standard bTB breakdowns relative to chronic breakdowns and (ii) herds which did not experience a bTB breakdown, relative to chronic herds.

MATERIALS AND METHODS

Data manipulation for the case-case and case-control analysis

A case-case analysis was devised to compare cases of either prolonged or recurrent bTB with cases of "standard" bTB breakdowns. A dataset of all confirmed bTB breakdowns spanning from 2003 to 2015 was made available from the NI Department of Agriculture, Environment and Rural Affairs (DAERA) database, the Animal and Public Health Information System (APHIS). Herds could appear more than once if they had experienced multiple breakdowns during the study period. Chronic breakdowns were defined as (i) prolonged (lasting longer than 365 days); or (ii) recurrent, (lasting less than 365 days), but followed by two or more further breakdowns within two years of the initial herd breakdown. This dataset also included 18 bTB breakdown-level predictor variables for consideration (Table 1).

Additional variables in Table 1 include those derived from *M. bovis* spoligotyping and genotyping of Variable Number of Tandem Repeat (VNTR) loci. Eight VNTR loci were sampled; MV2163B/QUB11B, MV4052/QUB26A, MV2461/ETRB, MV1955/Mtub21, MV1895/QUB1895, MV2165/ETRA, MV2163/QUB11A and MV3232/QUB3232. These were concatenated with the spoligotype to create a Multi Locus VNTR Analysis (MLVA) string, i.e. the strain. The final MVLA followed the naming convention "VNTR.spoligotype". Prior to 2009, strain typing was carried out only on the first reactor or lesion in a herd breakdown, whereas post-2009, each reactor was strain-typed. To correct for this, only one strain was selected from post-2009 breakdowns, using weighted probability based on the rates of occurrence of each strain in each breakdown. This MVLA was used to create the variable

MLVA_group. The 11 categorical values for this variable were derived from the top-ten most prevalent strains in the dataset: 2.142 [1 (reference-type)], 1.140 [2], 5.140 [3], 6.263 [4], 4.140 [5], 3.140 [6], 7.140 [7], 9.273 [8], 11.145 [9] and 49.140 [10]. These strains accounted for over 75% of all breakdowns, however a category called OTHER [11] was retained for breakdowns attributed to the remaining MVLA strains. The number of spoligotype and MVLA strains in an episode were also calculated.

Wildlife metrics were based on a 2007-2008 systematic survey of 212 1km^2 grid squares across NI (Reid et al., 2012), which was then modelled to achieve predicted relative abundance estimates for all of NI. These data suggested the badger population remained stable since a 1993-1994 survey, and were therefore considered representative for the study period. Furthermore, there has been no badger culling interventions during the study period. Along with survey data, estimates of the numbers of badgers per km^2 was previously derived using genetic data (Kostka, 2012). Information on road-traffic accident (RTA) badgers was included (Abernethy et al., 2011), specifically, whether the index herd experienced a breakdown where an RTA badger with a matching spoligotype or MVLA type was found within 3km of the herd, in either the years preceding, during or succeeding a herd breakdown.

Model Building

Data exploration at the univariable stage for both the case-case analysis and case-control analysis included generating summary statistics, cross tabulations and plotting smoothed scatterplots. Continuous variables were assessed for linearity in the logit and categorised into quartiles if found to be non-linear. In both analyses, the relationship between the response variable (i.e. chronic or non-chronic) was individually assessed against each explanatory variable with univariable Wald tests. Variables which exhibited $p < 0.20$ were retained for multivariable analysis. Following this, 19 out of 23 fixed-effect explanatory variables were retained for the case-case model, and all 14 explanatory variables were retained for the case-control model.

Generalized Linear Mixed Models (GLMMs) with a binomial distribution and a logit link function were fitted for both the case-case model and the case-control model. In both cases, the variables dvo_code and breakdown_year were included as random effects. Models were assembled using forward and backwards stepwise selection and the final models were assessed for congruency, whilst assessing for confounding and interactions. Better fitting models were selected using Likelihood Ratio Tests. The final models where assessed for co-linearity using the Variance Inflation Factor, retaining only the variables which showed the strongest association with the outcome variable. Goodness of fit was determined by Hosmer & Lemeshow goodness-of-fit tests during construction of preliminary Generalized Linear Models (GLM), and the model's discriminatory ability was determined using AUC value/ROC curves.

RESULTS

The final 15,636 records in the case-case analysis included 2,181 "chronic" episodes (13.94%), consisting of 1,763 herds which had experienced at least one chronic breakdown between 2003 and 2015 (18.20%). The final case-control analysis dataset contained 2,181 "chronic" episodes, matched to 2,181 records drawn from 2,143 herds which had never experienced a bTB breakdown. Table 2 shows the variables which were significant in both multivariable analyses for the case-case and case-control scenario. They were: herd_size, patch_prev_year, associated_herds and prop_ 365_days.

Table 1. The 26 breakdown-level explanatory variables under consideration.

Variable name	Description
herd_id[a]	Unique herd identifier
breakdown_year[a]	Year breakdown was identified
dvo_code[a]	Large-scale administrative boundary-Divisional Veterinary Office
start_no_of_reactors	Start number of reactors
LRS_flag	Whether a lesion was identified at routine slaughter
cc_flag	Whether the breakdown was confirmed via culture
herd_size	Number of cattle at time of breakdown
milklicence_flag	Dairy or non-dairy enterprise
associated _herds	Association to another herd via geography or management
patch_prev_year	Cattle bTB prevalence, where patch is a subdivision of DVO
av_patch_prev	Patch cattle bTB prevalence, averaged over the study period
herds_within_med_nn	No. herds within median nearest neighbour distance for NI herds
min_dst_closest_herd	Distance in metres of the closest herd
no_buy_ins	Herds which had never bought in cattle
prop_ 180_days	No. cattle bought in 6 months pre-breakdown, as % of herd size
prop_ 365_days	No. cattle bought in 1 year pre-breakdown, as % of herd size
high_turnover_herd	Herds where 30% of the total was sold in the year pre-breakdown
bought_in_bkdn_lstyr	bTB found in any herds which the index herd had purchased from
MLVA_group	The top-ten most prevalent strains in the dataset
n_spo_episode	The number of different spoligotypes present in an episode
n_MVLA_episode	The number of different MVLA types present in an episode
main_setts	The number of badger setts per km^2
badger_1	The number of individuals per km^2
badger_2	The number of individuals per km^2 (estimate via genetics)
RTA_badger_spo	RTA badger with a matching spoligotype within 3km of the herd
RTA_badger_MVLA	RTA badger with a matching MVLA within 3km of the herd

[a]Random-effect variables

Four additional variables were found to be significant in the case-case analysis: start_no_of_reactors: OR 1.02 (95%CI: 1.01–1.03); cc_flag: OR 0.16 (95%CI: 0.12–0.22); n_MVLA_episode: OR 3.79 (95%CI: 3.36–4.28); and the categorical variable MLVA_group. In the case-control analysis, additional significant variables were milklicence_flag: OR 3.20 (95%CI: 2.17–4.71); and herds_within_med_nn: OR 1.50 (95%CI: 1.25–1.80).

The wildlife reservoir

The wildlife abundance variables (badger_1, badger_2, and main_sett) were significant in the univariable context but exhibited substantial co-linearity. Therefore, only badger_1 was considered for inclusion in the multivariable context as it was obtained by systematic survey. Whilst the importance of badger_1 at the univariable level is clear for both the case-case: OR 1.07 (95%CI: 1.05-1.10) and the case-control: OR 1.55 (95%CI: 1.48-1.62, see Fig. 1) it did not contribute significantly to the final models and was therefore not included. Further investigation revealed that much of the risk posed by regional variation in badger abundance

was sufficiently captured in the spatial variables dvo_code and av_patch_prev. This was especially true for the case-case analysis. Here, a follow-up Poisson GLM model was not improved by the inclusion of the badger_1 variable, when dvo_code was the sole predictor of counts of chronic breakdown (χ^2 =1.03, df =1, p = 0.3; Fig. 2A). In the case-control scenario, some local effects of wildlife density were discernible within-DVO (Fig. 2B). The herd_size variable was so dominant in the dataset however, that a case-control model with herd_size as the only independent variable had an AUC of 0.89 (95%CI: 0.88 - 0.90) for predicting chronic breakdowns.

Table 2. The four variables from the case-case experiment and the case-control experiment found to be significant predictors of chronic breakdowns. Abbreviations are: av. pp = av_patch_prev, assc. hrds = associated_herds prp. 365 = prop_ 365_days.

	Case-Case			Case-Control		
Variable	Categories	OR	95% CI	Categories	OR	95% CI
herd_size [0]	1-40	-	-	1-10	-	-
herd_size [1]	41-85	1.59	1.34-1.88	11-100	2.15	1.78-2.51
herd_size [2]	86-175	2.21	1.86-2.61	101-200	5.71	4.78-6.82
herd_size [3]	176-1412	3.23	2.71-3.85	201-1412	19.14	14.67-24.97
av. pp [0]	3.18-7.80	-	-	0-6.54	-	-
av. pp [1]	7.81-10.28	0.96	0.82-1.12	6.71-8.54	3.31	2.36-4.64
av. pp [2]	10.34-13.31	1.15	0.98-1.34	8.55-11.40	5.77	4.00-8.31
av. pp [3]	13.47-25.43	1.20	1.02-1.41	11.61-25.43	19.05	12.71-28.54
assc. hrds	-	1.31	1.16-1.47	-	2.55	1.87-3.48
prp. 365 [0]	0-1	-	-	0-1	-	-
prp. 365 [1]	2-10	0.86	0.74-1.01	2-3	3.59	2.26-5.69
prp. 365 [2]	11-50	1.05	0.91-1.21	4-30	4.22	3.04-5.86
prp. 365 [3]	51-100	1.49	1.29-1.71	31-100	12.34	8.80-17.32

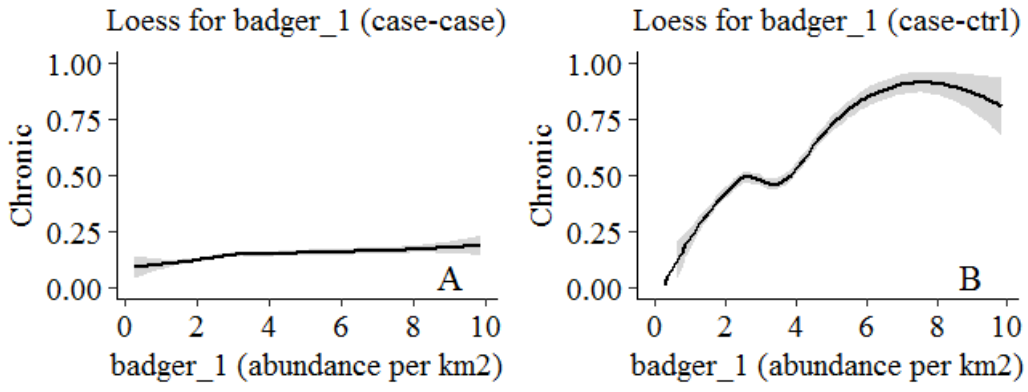

Fig. 1 LOESS (locally weighted smoothing) curves for badger_1 in the case-case analysis (A) and the case-control analysis (B).

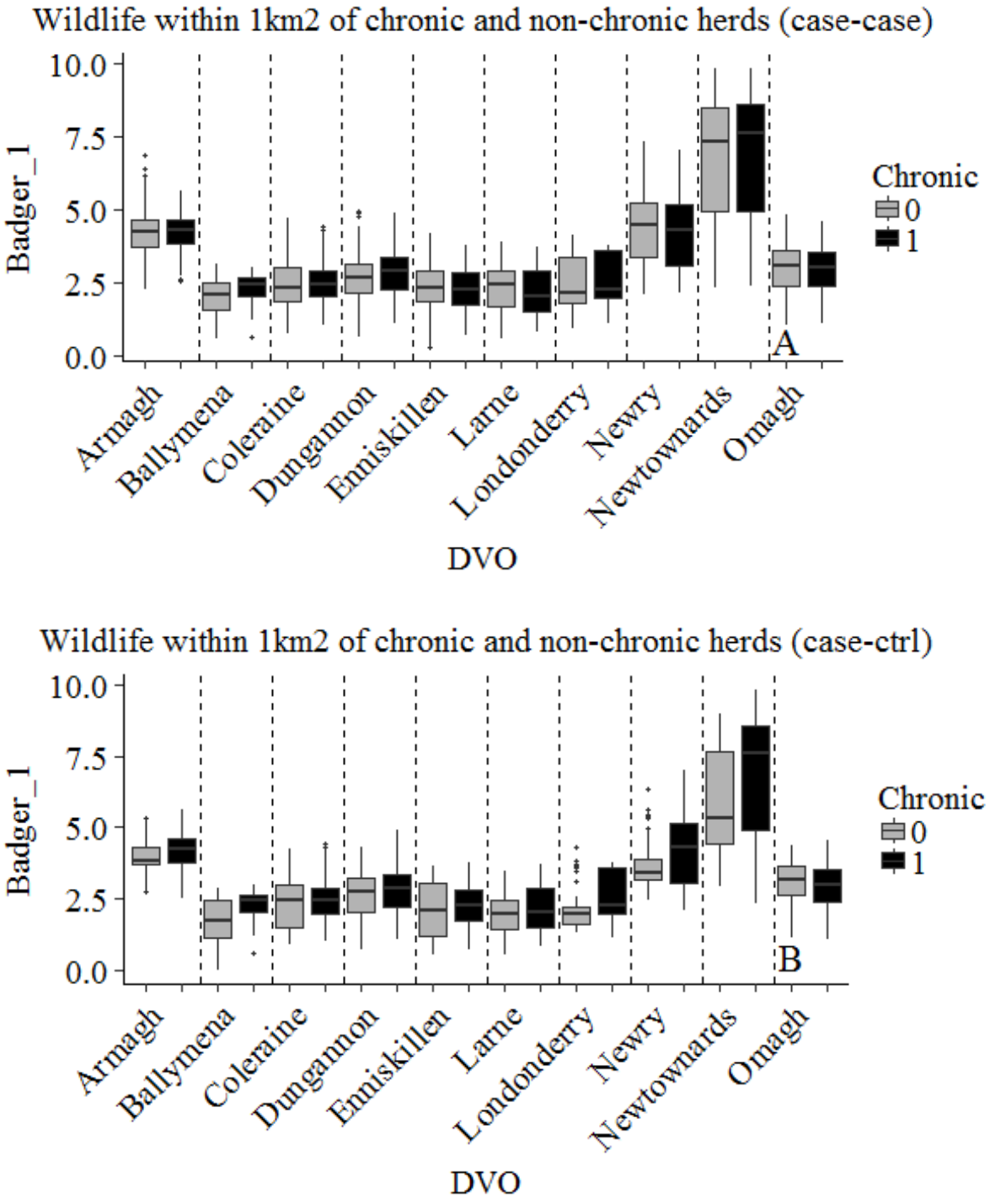

Fig. 2 Boxplots showing the distribution of chronic and non-chronic breakdowns in relation to badger abundance data in each DVO for the case-case (A) and case-control (B).

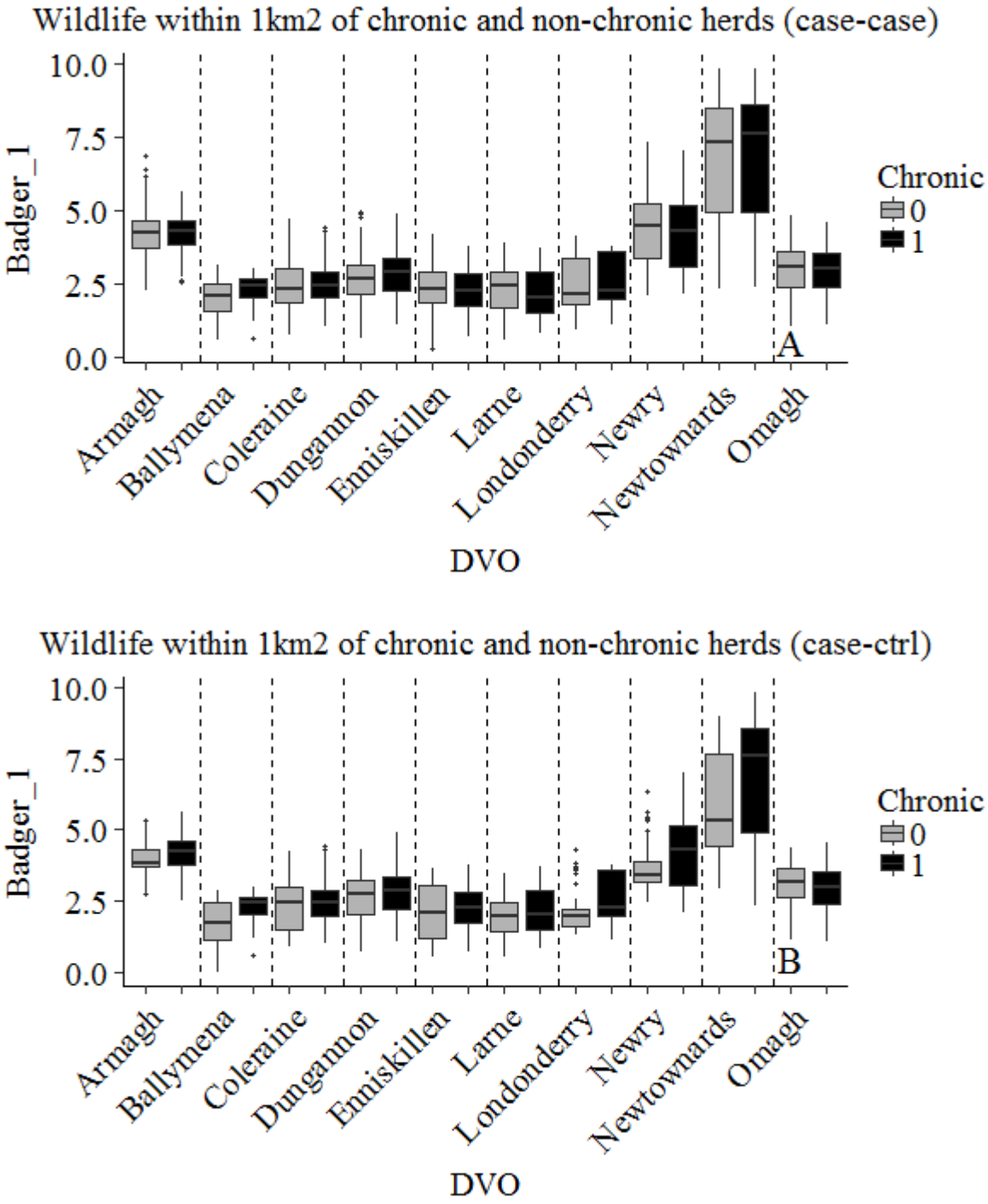

Fig. 2 Boxplots showing the distribution of chronic and non-chronic breakdowns in relation to badger abundance data in each DVO for the case-case (A) and case-control (B).

The effect of bTB strain

In both the univariable and multivariable case-case context, different *M. bovis* strains were associated with different levels of chronic breakdown risk. When comparing the final case-case model to a model including an interaction term between dvo_code and MLVA_group, no

improvement in model fit was achieved. It was therefore determined that this effect was independent of the administrative Divisional Veterinary Office boundaries.

The comparative risk associated with different strains was assessed using multiple comparison procedures via a post-hoc Tukey HSD test, carried out on the final case-case model. The results for the strains which exhibit a significant comparative difference in risk are shown in Table 3, and the estimates for risk and family-wide confidence intervals for all of the results, including comparisons with non-significant differences, are shown in Fig. 3. These data indicate that a number of strains appear to differ in their risk profile when compared to other strains. Notably, strain 3.140 [6] was associated with significantly increased risk when compared to strains 2.142 [1], 5.140 [3], 9.273 [8], 11.145 [9], 49.140 [10] and OTHER [11]. Strain 4.140 [5] was also associated with increased risk compared to strains 2.142 [1], 5.140 [3], 9.273 [8], 11.145 [9] and OTHER [11]. Additionally, strain 9.273 [8] was associated with decreased risk, when compared to 1.140 [2], 6.263 [4], 4.140 [5] and 3.140 [6].

Table 3. Pairwise comparisons of the risk of the 10 most prevalent strains of bTB found in NI (Strain 1) relative to a comparator strain (Strain 2). Only those strains which exhibited a significant difference in risk are presented.

Strain 1	Strain 2	Estimate	Std. Err.	Z-statistic	P-value	OR
2.142 [1]	6.263 [4]	-0.34	0.104	3.31	0.03	0.71
2.142 [1]	4.140 [5]	-0.51	0.104	4.96	<0.01	0.60
2.142 [1]	3.140 [6]	-0.58	0.117	4.96	<0.01	0.56
1.140 [2]	9.273 [8]	0.63	0.187	-3.36	0.03	1.88
5.140 [3]	4.140 [5]	-0.46	0.117	3.96	<0.01	0.63
5.140 [3]	3.140 [6]	-0.53	0.131	4.04	<0.01	0.59
6.263 [4]	9.273 [8]	0.72	0.195	-3.72	<0.01	2.05
6.263 [4]	OTHER [11]	0.32	0.099	-3.27	0.03	1.38
4.140 [5]	9.273 [8]	0.89	0.195	-4.59	<0.01	2.44
4.140 [5]	11.145 [9]	0.65	0.205	-3.17	0.05	1.92
4.140 [5]	OTHER [11]	0.5	0.099	-5.00	<0.01	1.65
3.140 [6]	9.273 [8]	0.96	0.203	-4.75	<0.01	2.61
3.140 [6]	11.145 [9]	0.72	0.212	-3.4	0.02	2.05
3.140 [6]	49.140 [10]	1.01	0.312	-3.23	0.04	2.75
3.140 [6]	OTHER [11]	0.56	0.115	-4.92	<0.01	1.75

DISCUSSION

These data reveal that four key variables were implicated in chronic breakdowns both when assessed against “standard” bTB breakdowns, and also compared with herds which have never broken down. These were herd size, average patch prevalence for bTB, the presence of associated herds and the movement intensity in the year preceding breakdown.

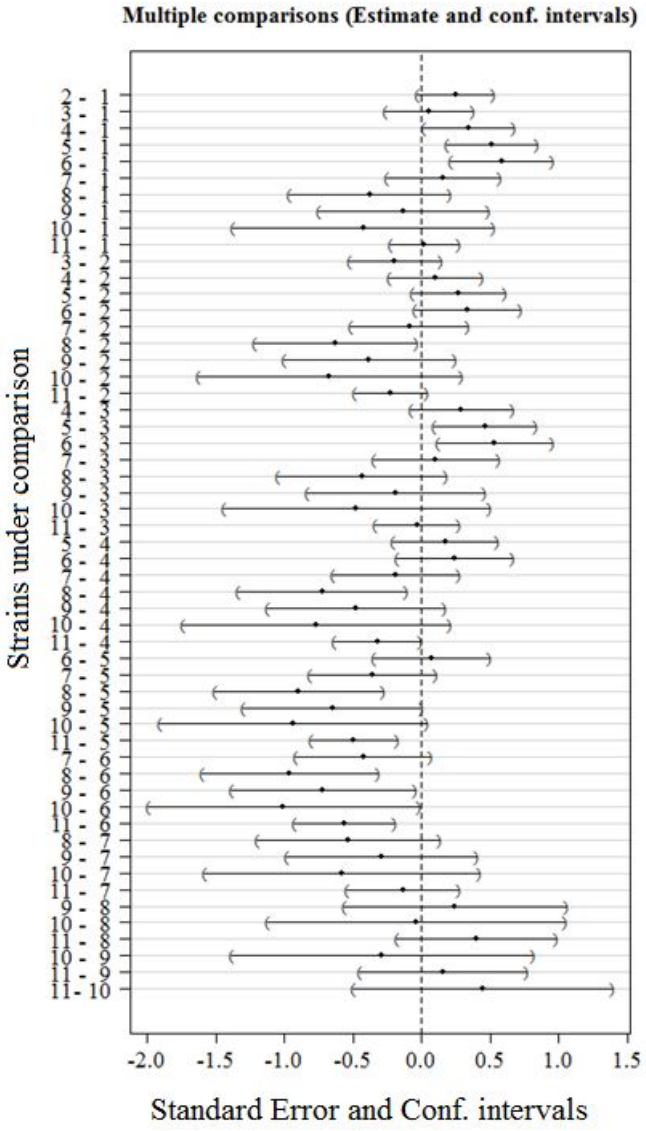

Fig. 3 Estimates of risk and family-wide confidence intervals between the 10 most prevalent strains. The naming convention of the strains along the y-axis follows the materials and methods; 2.142 [1 (reference-type)], 1.140 [2], 5.140 [3], 6.263 [4], 4.140 [5], 3.140 [6], 7.140 [7], 9.273 [8], 11.145 [9] 49.140 [10] and OTHER [11].

These variables, amongst others, were also identified by Doyle et al. (2016) in an analysis of chronic breakdowns in NI. Herd size is a known risk-factor for bTB breakdowns generally (Reilly & Courtenay, 2007; Brooks-Pollock & Keeling, 2009), possibly because larger herds may simply be more likely to harbour a reactor. Large herds may also be associated with increased agricultural intensity or a larger geographic footprint leading to increased contact with other sources of infection (e.g. wildlife or contagious farms). Alternatively, increasing herd size in conjunction with low SICCT test sensitivities may lead to failure to fully clear infection and thus drive disease persistence within the herd. Herd associations may represent shared grazing with infected cattle, or sharing of contaminated facilities and equipment, leading to persistence of bTB and failure to clear infection. High inwards movement intensity (Gilbert et al., 2005; Wolfe et al., 2009) may result in the continuous introduction of infected cattle,

leading to an increased number of breakdowns, or perhaps prolong breakdown length. High levels of patch bTB prevalence associated with chronic breakdowns indicates that local environmental persistence is a contributing factor, perhaps via wildlife or neighbouring farms (White et al., 2013).

Chronic breakdowns are associated with increasing *M. bovis* strain diversity, and these strains include those known to the area, along with ones from outside of their "home ranges" (Skuce et al., 2010). This suggests that multiple sources, including bought-in cattle, wildlife, lateral spread and local environmental persistence, are all implicated in chronic breakdowns. Critically, different strain types were associated with different levels of risk of chronic breakdown. Although subtle, these findings are concurrent with previous work by Wright et al. (2013b), who found that some molecular types, notably strains 3.140 and 4.140, were associated with lower numbers of lesioned skin test reactors compared to other strains, although the results lacked statistical significance. Here, these two same strains (3.140 and 4.140) were found to be associated with more chronic breakdowns than others. Furthermore, strain 9.273 was associated with a greater number of lesioned reactors (Wright et al. 2013b), and here strain 9.273 is associated with decreased risk of chronic breakdown. Whilst this may suggest that different strains may be harder to detect and eradicate thus leading to persistent infections, more work is required to validate this line of enquiry.

Analysis of the wildlife density data found positive associations between 1 km^2 badger density and chronic breakdown risk at the univariable level, however in the multivariable context, the regional differences in bTB risk posed by wildlife density were adequately captured by other larger-scale spatial variables such as DVO. Additionally, there is a stronger association between wildlife density and chronic breakdown risk in the case-control model than in the case-case model. Whilst an association with increasing wildlife density between herds which have never experienced a bTB breakdown and those chronically infected is unsurprising, the risk presented by wildlife is therefore not completely clear in the context of the case-case analysis i.e. between "standard" bTB breakdowns and "chronic" bTB breakdowns. The authors' therefore suggest that higher badger populations help maintain patch bTB prevalence, and may also be implicated in spillback infection in areas with higher levels of infected badgers. However, badger density may not be as an important risk factor for longer term maintenance of infection once *M. bovis* is introduced to a herd, where within-herd transmission may be the predominant factor. This is concurrent with research which has verified an association between the presence of wildlife and bTB breakdown risk (Bessell et al. 2012; Wright et al. 2015). Alternatively, given the variation in badger bTB risk (Byrne et al. 2015) and the potential for frequency-dependent transmission between hosts, alternative spatial models capturing infection risk of badgers (as opposed to density *per se*) may reveal further insights into the transmission dynamics between wildlife and domestic hosts.

ACKNOWLEDGEMENTS

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IMPROVING DISEASE CONTROL

NUDGING TO IMPROVE COMPLIANCE WITH ANIMAL HEALTH POLICIES: AN INVENTORY OF APPROACHES CONSIDERED BY ANIMAL DISEASE MANAGERS

M. GARZA, E.C. ÅGREN AND A. LINDBERG*

SUMMARY

Strategies for provision of information, used in animal health surveillance and control programmes, and aimed at influencing behaviours such as programme enrolment, adopting certain biosecurity behaviours, engaging in surveillance or complying with an activity were identified through interviews with professionals from seven European countries. Theoretical frameworks from nudge theory (EAST and MINDSPACE) were applied with the purpose of targeting strategies that were well-designed from a theoretical perspective and describing what psychological mechanisms they activate. It was concluded that almost half of the strategies were designed in a manner that fulfils existing recommendations for successful uptake of policies, and includes multiple triggers of psychological mechanisms that support conscious or intuitive actions. It is suggested that it would be beneficial to consider randomised controlled trials or retrospective cohort studies to assess the value of identified strategies, and in the future, to include such assessments in the design of surveillance and disease control activities.

INTRODUCTION

Nudge theory is a concept in behavioural science, political theory and economics which argues that positive reinforcement and indirect suggestions to achieve non-forced compliance can influence the motives, incentives and decision making of groups and individuals as effectively as direct instruction, legislation, or enforcement (Thaler & Sunstein, 2008). It has previously been used in other fields, for example to promote environmentally sustainable behaviour (Castelo, 2012) and to help implement public policies (Haynes et al., 2012), but its usefulness in supporting animal health policies is still to be explored (Barnes et al., 2015).

Animal health surveillance and control activities are usually designed with the assumption that all actors are fully compliant, meaning there may be assumptions regarding sample sizes and risk behaviour that are unrealistic. Deviations from these assumptions, i.e. non-compliance, may impact on the cost-effectiveness of risk-based surveillance strategies, where loss of

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information from high risk strata may significantly reduce surveillance sensitivity. It may also lead to underestimation of introduction risks or detection probabilities.

Activities where participation is voluntary or requires complex actions are the most vulnerable to non-compliance (Brugere et al., 2017). Therefore, surveillance designers often use so called 'enhancements' to mitigate the risk of non-compliance by promoting the desired behaviour in different ways. For passive surveillance activities, this could, for example, be in the form of financial rewards for notifications, training to increase awareness and recognition of clinical signs, awareness campaigns to improve recognition of disease and awareness of reporting obligations/procedures, payment of compensation for mitigation measures, provision of alternative routes of reporting such as a phone hotline or notification by SMS, subsidised testing cost or some other form of benefit, such as farmers receiving advice in return (www.fp7-risksur.eu/terminology/faq). In a voluntary control programme, similar types of design features have been used to promote the affiliation to and uptake of control strategies, for example by peer influence (Hult & Lindberg, 2005). These design features fall into the definition of nudges, but so far have not been assessed against nudge theory, nor scientifically validated or justified as to whether they are effective. Furthermore, such operational features often form part of professionals' individual experience of implementation of disease control, and tend not be reported in the scientific literature and official documents, making it difficult to identify them by systematic literature reviews.

Several theoretical frameworks exist in nudging literature, and they capture somewhat different aspects related to behavioural influence. Examples are: the Nuffield Ladder of Intervention (Nuffield Council on Bioethics, 2007), which describes the relationship between the strength of an intervention and freedom of choice; MINDSPACE, developed by the UK Institute for Government, which provides a checklist of psychological approaches to apply when seeking behavioural change (Dolan et al., 2012); and the EAST framework, which focuses on design elements related to the successful uptake of interventions (Service et al., 2015). Additionally, Ly and co-workers (2013) have presented a generic framework for categorising nudges, irrespective of how they are implemented.

This study was conducted as part of the ANIHWA-funded SANTERO (risk-based Surveillance for ANimal healTh in EuROpe) project (2016-2017) that aimed to promote further development of risk-based surveillance methods and provide support for their dissemination and integration in existing surveillance routines (santero.fp7-risksur.eu). The objective was to carry out an inventory of animal health surveillance and disease control strategies and designs that have been implemented in the past, and that serve to improve acceptance of, and compliance with, a particular surveillance and/or control activity. More precisely, the study aimed to: 1) identify design features in current and past animal health surveillance and control programmes that aim to influence behaviour; 2) classify and describe them according to nudge theory; and 3) discuss their suitability for testing in intervention studies.

MATERIALS AND METHODS

Study participants

Professionals with practical experience in designing and implementing surveillance and control programmes, from seven countries in Europe (Sweden (SE), the Netherlands (NL), Switzerland (CH), Norway (NO), Denmark (DK), Ireland (IE) and the United Kingdom (UK)), were recruited via the network of surveillance experts involved in the SANTERO Consortium.

The selection of participants was purposive and followed a snowballing sampling approach. SANTERO partners were asked to provide initial suggestions of suitable experts from their countries. These were subsequently contacted, and if agreeing to participate they were further asked to advise on other professionals to interview, who matched the desired population. Some participants also suggested additional names without being asked. In all, 37 experts were contacted, and 28 accepted participation.

Data collection

An interview protocol was developed with the aim of capturing design features meant to facilitate voluntary expression of desired behaviours during the implementation of animal health surveillance and disease control activities. Desired behaviours include e.g. enrolment in a control programme, engagement in surveillance activities, compliance with the rules and regulations of animal health programmes or adoption of certain biosecurity practices. Other contextual information considered relevant was also included in the protocol, such as information about the respondent's role and past or current involvement in animal health programmes. Examples of strategies were provided in the protocol to aid understanding of the scope of data collection.

The interviewees came from different fields (livestock, wildlife, aquaculture) and the interview openly asked about strategies applied within context, and in relation to their specific target groups; primarily farmers, hunters and the general public.

In the light of diversity in respondent expertise, the scope was widened to allow additional examples from the field of animal health management, as well as statutory activities. In addition, although livestock producers, hunters and the general public were the main target groups, strategies directed towards professionals with other roles in relation to animal health were also considered.

The protocol was shared with the respondents after they had agreed to participate, but before the interview took place. The interviews were conducted in person, by telephone or by online meeting applications in April and May 2017, in English. They lasted between 45 minutes and one hour and were recorded to facilitate recollection.

Data editing

Extracts of interest were transcribed, anonymised, and checked for accuracy. Transcripts were then screened with special focus on information about strategies with potential behavioural influence, with the aim of identifying the desired or anticipated behaviour underlying the strategy, implementer, stakeholder to influence and other characteristics such as type (surveillance activity, control scheme, general biosecurity programme, biosecurity measure or general animal health management), whether the strategy was voluntary or compulsory, species, pathogen or disease and the country of implementation. The (perceived) outcome of the strategy was also noted, in case this was reported. The data were entered in Microsoft Excel 2016, for further cleaning and analysis.

The variables extracted from the data were categorised to facilitate analysis. Firstly, desired behaviours were categorised into: "enrol and engage in a programme", such as showing interest in an animal health activity, or enrolling or actively proceeding to a higher level in a biosecurity programme; "engage in surveillance", including e.g. submitting samples or reporting dead

animals; “comply with surveillance and control activities”, i.e. following stated rules and regulations, irrespective of whether they are compulsory or not, and irrespective of the legal basis, such as the culling of persistently infected (PI) animals, trade with the same, or a better, category of animals; and finally, “adopt certain biosecurity practices”, such as performing a proper biosecurity behaviour on the farm or in conjunction with transport.

Implementers were categorised as “animal health services”, “industry”, meaning the policy level rather than individual companies, and “authorities”, referring to authorities in charge of surveillance, programmes or sampling. Similarly, stakeholder to influence was classified into producers, hunters, general public, animal health providers, industry stakeholders, authorities, and other groups such as farm- and abattoir workers, transporters or traders, or a combination.

Data analysis

Application of frameworks: The strategies were filtered and described according to three theoretical frameworks, namely the Nuffield Ladder of Intervention, EAST and MINDSPACE.

Firstly, the different strategies were categorised according to the Nuffield ladder of intervention which reflects the degree of intrusiveness of a policy, ranging from the least intrusive levels: “Do nothing” and “Provide information”, to the strongest: “Eliminate choice”. Potential nudges would, by definition, be found at the lower, non-restrictive, levels. For the purposes of this paper, the dataset was delimited to strategies that fell within the lowest category “Provide information” only, some of which also matched the category “Enable choice”. This means that strategies guiding choice through a change in the default policy, by incentives or disincentives, are not included.

Subsequently, the remaining strategies were contrasted against the EAST framework, which is a mnemonic that stands for *Easy, Attractive, Social* and *Timely*. These are all attributes that characterise approaches with high potential to engage and activate people, thereby triggering desired behaviour, or behavioural change. *Easy* focuses on how the strategy makes it easier for the individual to perform a desired behaviour, e.g. by framing information in a simple manner, by using default options to reduce effort, or by making it easy to carry out the desired action, such as submitting samples or signing up for a programme. *Attractive* means that the strategy should be designed so that it is desirable to perform the behaviour. This can be by attracting attention, e.g. by personalising the information, or using social or individual reward systems, such as providing a positive self-image. Examples are animal health schemes that involve scores and tests, or public availability of herd status on the web or by a sign on the entrance door of the barn. In addition, strategies have a stronger behavioural influence when they take place in a *Social* context. This could be achieved by setting social norms, and showing what others do, such as referring to how many other farmers have joined a programme, or have herds free of a disease. The use of relationships within networks has a similar effect. Commitments also make strategies social, such as publicly signing up for an animal health programme. Finally, strategies should be applied in a *Timely* manner. Accordingly, individuals should be approached in situations when they are more receptive, due to convenience or because it is more favourable for them to carry out the behaviour. Sending reminders to farmers to cull PI animals before opportunities to receive subsidies expire, is an example of such timeliness.

The EAST framework was used to identify combinations of attributes considered in strategy design. To capture strategies that, at least theoretically, have a high likelihood of being successful, further focus was given to those that incorporated three or more of the EAST

attributes. These were subsequently described by means of the MINDSPACE framework, to obtain insight into the potential psychological mechanisms involved when applying the strategy.

MINDSPACE provides a perspective that is complementary to EAST, and captures nine different psychological mechanisms by which behaviour can be influenced. These are denoted *Messenger, Incentives, Norms, Defaults, Salience, Priming, Affect, Commitments* and *Ego*. Briefly, the *Messenger* mechanism is based on how humans respond to information from different sources, and has to do with who is found trustworthy and credible. In the animal health context for example, a farmer might react more positively/engage more when a respected colleague with experience of the disease in question, speaks about the value of enrolling in a programme, in comparison to other sources of information. *Incentives* act upon mental shortcuts, so called "system 1 thinking" (Morewedge & Kahneman, 2010) and are geared towards avoiding losses. The effect of incentives is known to vary according to type, magnitude and timing. Examples include subsidies and payments to undertake diagnostic investigation of differential diagnoses, in case a suspicion of exotic disease tests negative. *Norms* are behavioural expectations or rules within a group, and reflect the fact that information about how others behave can influence an individual's behaviour, particularly if expressed at the group level. Hence, providing information to farmers on what others do, and/or capitalising on relationships with other individuals in associations or networks to implement strategies, could be examples of this mechanism. *Defaults* have an effect when strategies are designed to make the unwanted option more difficult to choose, while facilitating options that lead to a desired behaviour. An example is the use of active opt-out, which can be applied when seeking permission to use clinical records for secondary purposes (such as surveillance, or research), by asking animal owners to return a form by mail if they want to opt-out, where the default would be to accept with no action required. The *Salience* mechanism triggers the human inclination to turn attention to important things that can be related to; these are likely to be novel, accessible and simple. Losses are also, in general, more salient to us than gains. *Priming* has the effect of increasing the likelihood of performing a desired behaviour after exposure to certain stimuli that are not consciously processed. One example is when information about a control scheme is communicated in positive terms some time before potential programme participants are approached. *Affect* is based on the power of generating emotions, through words, images or events. According to this mechanism, biosecurity messages are more likely to be adopted if they are framed in dramatic terms. Some strategies promote *Commitment* of farmers by using contracts or by making their herd health status public, since individuals try to be consistent with public promises and reciprocal acts. Finally, the *Ego* mechanism comes into play when strategies prompt a positive self-image, making individuals feel better about themselves, such as producers wanting to advance in control programmes, or hunters participating in surveillance of wildlife diseases with public health impact, to compensate for an otherwise potentially negative public image. Unlike EAST, MINDSPACE was not used to filter for potentially more effective and successful strategies, but rather to provide in-depth behavioural insights to strategies identified as potentially effective by EAST. A matrix was developed to link the interpretation of EAST attributes to MINDSPACE mechanisms (data not shown).

Statistical analysis: Descriptive statistical results were produced from covariates extracted from interviews. Furthermore, associations between type of activity versus desired behaviour and implementer were tested for, as well as between, individual- and cumulative number of EAST attributes versus activity and implementer, respectively. Contingency tables were generated, and homogeneity of distributions was examined with Fischer´s exact tests, noting

all associations with a p-value <0.10; liberally chosen considering the limited sample size and indicative nature of this study.

RESULTS

Descriptive statistics

A total of 126 strategies were provided in 27 interviews, of which 5 resulted in more general information or information repeated from that already collected. Delimiting the data mainly to records that, according the Nuffield ladder of intervention, could be classified as "Providing information", resulted in 51 remaining strategies. General characteristics of these data are summarised in Table 1.

Table 1. Overview of the context within which strategies aimed at influencing behaviour using provision of information have been implemented in animal health activities in seven European countries.

Country	No. of interviewees	No. of activities	No. of strategies	Species involved	Disease or pathogen[a]
Denmark	2	5	8	Cattle, mixed farm animals	Johne's Disease, *Salmonella* Dublin, general AH
Ireland	1	1	8	Cattle	BVD
Netherlands	3	4	6	Poultry, aquaculture, mixed	Notifiable diseases[b], endemic diseases, aquatic bacterial zoonoses
Northern Ireland	1	2	7	Cattle, mixed farm animals	BVD, general AH
Norway	3	6	8	Aquaculture, pigs, wildlife	General AH, ISA, CWD, MRSA, *Mycoplasma hyopneumoniae*, notifiable diseases
Sweden	4	3	9	Cattle, wildlife	General AH, BVD, *Echinococcus multilocularis*
Switzerland	2	5	5	Cattle, wild birds, bees	Beetle bee, BVD, AMR, BSE, AI
Total	16	26	51		

[a]AH: Animal health; BVD: Bovine viral diarrhoea; AI: Avian influenza; AMR: Antimicrobial resistance; BSE: Bovine Spongiform Encephalopathy; CWD: Chronic Wasting Disease; ISA: Infectious Salmon Anaemia; MRSA: Methicillin resistant *Staphylococcus aureus*

[b]Notifiable diseases: Avian Influenza, Newcastle disease, African Swine Fever, Classical Swine Fever, Foot and Mouth Disease, Schmallenberg, Equine Herpes Virus among others

The interviewees jointly reported between five and nine information provision strategies per country. The most common context mentioned was control programmes for endemic diseases in cattle (n=24), followed by surveillance activities for notifiable diseases (n=12).

The association between behaviour of interest to influence ("desired behaviour") and activity in question was significant (p<0.001), with enrolment being a frequent objective for strategies in control schemes and, to some extent, compliance, whereas engagement was the most frequent goal for strategies used in surveillance activities (Table 2). Almost two thirds of

strategies (n=32) were seen in activities implemented by animal health services, whereas authorities were responsible for implementation of almost a third of strategies (n=16). Further, the media was mentioned a few times (n=2) as a source providing information about surveillance activities run by authorities, mainly for diseases of public health impact. A clear pattern was seen when looking at type of activity and implementer ($p<0.001$), with authorities being responsible for surveillance of notifiable diseases, whereas animal health services are highly responsible for implementation of control programmes.

Table 2. Type of activity and implementer of strategies aimed at influencing behaviour, in relation to the desired behaviour they intend to promote. The material is limited to strategies primarily focusing on provision of information within animal health surveillance and control activities in seven European countries.

	Categories	N	Adopt certain biosecurity practices	Enrol and engage in pro-gramme	Engage in surveil-lance activities	Comply with an activity or programme
Activity	Animal health management	5	0	0	5	0
	Biosecurity training	2	2	0	0	0
	Biosecurity programme	3	0	1	0	2
	Control scheme	27	0	18	0	9
	Surveillance	14	0	0	10	4
	Total	51	2	19	15	15
Imple-menter	Animal health services	32	1	17	4	10
	Authorities	16	0	2	10	4
	Media	2	0	0	1	1
	Missing	1	1	0	0	0
	Total	51	1	19	15	15

Application of the EAST framework

Table 3 illustrates patterns of attribute combinations obtained by applying the EAST framework. Of 51 strategies, 21 (41%) incorporated three attributes, whereas only one clearly used all four. The most common combination captured *Attractive, Social* and *Timely*. Nineteen strategies used two attributes; of these, the more common combinations were *Attractive* and *Social*, or *Attractive* and *Timely*. In all, 40 strategies included features that made it *Attractive* to perform a desired behaviour. There was a significant association with type of activity ($p=0.085$), with most strategies for surveillance (13 of 14) showing this attribute. Type of activity was also significantly associated with the total number of EAST attributes incorporated in strategies ($p=0.086$), with surveillance activities and general biosecurity programmes having a higher observed number than expected by chance alone. The least exploited attribute overall, in this animal health surveillance and control context was *Easy*.

Strategies categorised as not including any EAST attributes were indirect/passive, and described as communication of disease occurrence to authorities, communicated to stakeholders at a later stage, with knowledge raising activities implemented using literature and farmer press.

There was no association between individual EAST attributes and implementer, nor between the number of EAST attributes and implementer.

Table 3. Combinations of attributes used (dark grey) in strategies aimed at influencing behaviour in implementation of animal health activities, based on provision of information.

	Easy	Attractive	Social	Timely	No. of strategies
					4
					3
					2
					1
					8
					7
					2
					1
					1
					17
					1
					3
					1
Sum	10	40	32	29	
Total					51

Application of the MINDSPACE framework

Of the 21 strategies that captured three of four EAST attributes, eight were aimed at voluntarily *enrolling or engaging people in a control programme*. For most, emphasis was placed on the context in which information was delivered (social and timely), and by framing messages in an attractive way. For example, experts from IE and NL reported delivering talks at agricultural shows/markets using animal health services staff who run control programmes. The aim was to attract farmer attention, using information about the benefits of schemes and costs of disease. Such strategies incorporated at least four MINDSPACE mechanisms; *Messenger, Norms, Salience* and *Priming*. Presenting positive information in a conducive social context builds on the idea that farmers become more receptive when approached for

enrolment at a later stage. This strategy also capitalises on potential social and normative effects of delivering information to a group sharing similar characteristics. In this context, the animal health services were described by interviewees as being a positively regarded information provider.

Furthermore, among strategies capturing three EAST attributes, farmers shared testimonies on the impact of diseases and positive experiences (e.g. when enrolled in a scheme) in conjunction with social events (markets etc.). In general, farmers acting as messengers, sharing experiences, were strategies used in almost all countries. This type of "ambassadorship" was described by study participants as well received by farmers, in particular, if the messenger farmer had a good community image and strong position. Farmers had also volunteered as pioneer ambassadors in early stages of control/eradication schemes, and actively tried to convince reluctant farmers to join. From a MINDSPACE perspective, mechanisms like *Messenger, Norms, Salience, Affect* and *Ego* played a role in these strategies.

The only strategy that clearly included all four EAST attributes was an enrolment strategy reported from SE, in which the Chief Veterinarian, regionally responsible for implementation of the BVD eradication scheme (employed by the regional dairy association) on the Isle of Gotland, invited dairy farmers, grouped according to probable BVD status, to lunches. Here, they received information about the scheme and the disease. Knowledge on BVD status was available for all dairy farmers based on results of national bulk milk screening conducted before the scheme started. This strategy included effective targeting and personalisation of information, in a conducive social environment, putting farmers at comfort and ease. At the end of the lunch meeting, an enrolment list was circulated. In this way, most MINDSPACE mechanisms were actioned: a trustworthy *Messenger*; a message and context possibly involving *Salience* and *Affect*; a social situation playing on *Norms*, *Commitment* and *Ego*, i.e. by seeing their peers signing up, farmers were inclined to follow, and/or due to the sense of reciprocity, they wanted to maintain a good image. An additional feature, was that enrolment lists included all farmers that were invited, so attendees could see who had not attended. Reportedly, attending farmers later approached non-attending peers with the message, possibly *Priming* them to later sign up.

The use of an interactive IT system to engage farmers in a voluntary industry biosecurity programme, aimed at promoting good animal health management, was also described. Its first stage is meant to lead to further engagement and is designed to be *Easy, Attractive* and *Social*. A main psychological mechanism in this respect is *Commitment*, as although voluntary, the programme involves a formal subscription and fee payment. Farmers then assess their own routines and practices using a test, before progressing onwards. Results are fed back as a score in a traffic light system. To prepare for the test, an online information platform is available, with content described using videos of other farmers explaining their experiences and views, animations on salient biosecurity risks, and other material to attract farmer's interest in programme progression. The first stage has a credible *Messenger*, informs about what others do (*Norms*), acts on *Salient* beliefs and possible *Affect*, involves *Commitment* and boosts farmer's *Ego*. In this way, programme progression is attractive for farmers, even when requiring more engagement, costs and involvement of a veterinarian.

Seven of the 22 strategies incorporating three or more EAST attributes promoted *engagement in surveillance activities*. In CH, media campaigns were used to convey information about Avian Influenza (AI) surveillance and encourage the general public to report wild bird mortality events and submit samples. The campaign used wildlife guards as

trustworthy *Messengers,* and also activated *Norms*, *Salience and Ego* as these officers are highly regarded, a source of clear and reliable information and have large 'social capital' (compared to authority figures or veterinarians). Another example was engagement of hunters in surveillance of *Echinococcus multilocularis* in SE, where authorities used hunter social networks to broadcast information and promote submission of faecal samples from foxes for national screening, emphasising the importance of their contribution to society (*Salience* and *Norms*). A symbolic compensation was paid (*Incentive*), but not to individual hunters; instead the fee went to the local hunters' clubs. This could positively affect engagement, potentially triggering hunters' sense of *Commitment* (to the club, and to society), and a positive self-image (*Ego*). A positive image and justification of hunting as an activity could also have played a normative role, particularly, as hunting is controversial. In subsequent stages of the surveillance programme, when participation decreased, the authorities tried to improve the attractiveness and timeliness of their information by using a mailing list to deliver updated and key information through the hunter network. The same network was used to transmit pre-hunting-season reminders about the importance of passive surveillance and availability of free post mortem services for wildlife; information that is *Salient* to hunters, possibly *Priming* them to act upon opportunities.

Some strategies were used to engage people in surveillance activities, targeted at time-points when they are more receptive; similar to enrolment strategies for endemic disease control programmes. In general, novel or unexpected events with high impact (e.g. exotic disease outbreaks) were reported from all countries as an opportunity for stakeholder engagement with surveillance activities, due to their *Salient* nature. Such events are also perceived to generate enhanced receptiveness for information on biosecurity and animal health management, in general. This phenomenon was seen across all production systems in the study. For example, during the onset of AI outbreaks in NL, the "ambassador" strategy was used, with farmers previously suffering disease serving as credible information *Messengers* to others within their network, reducing fear of economic and social consequences, and enhancing willingness to report events. Strategies to reduce fear of negative consequences were commented upon by a Dutch participant as being particularly important to implement in a timely manner to avoid misinformation, "since negative information tends to travel faster and spreads more widely than positive stories".

Seven strategies were found that aimed to improve *compliance with surveillance and control activities.* These displayed three EAST attributes (*Attractive, Social, Timely*), and aimed to improve uptake of voluntary but strongly recommended activities within control schemes during their compulsory phase, but also in advanced stages of industry-run biosecurity programmes. (In this context, "Providing information" was often combined with stronger interventions such as guiding choices through incentives or disincentives, which is out of scope for this paper). These strategies were often found to build on interaction between the veterinary practitioner/advisor, and farmer, in a "coaching" fashion. In this setting, personalised information and advice can be provided, and planning and implementation is more participatory, to facilitate farmer engagement. One example comes from the compulsory phase of the BVD scheme in IE, where culling of PIs is a strongly recommended measure, but nevertheless voluntary (no legal requirement). The 'nudge' is a timely text message (SMS), whereby farmers receive reminders to remove PI animals at key time-points. However, the effectiveness of this strategy also builds on a solid relationship with veterinary practitioners, supported by a targeted advisory IT system. The practitioner is involved in all testing and tissue tag procedures, and can provide encouraging advice in conjunction with such interactions. The involvement of the farmer is needed, as they report when required visits have been conducted.

Also, monitoring data are used to establish most likely route of infection, and inform tailoring of biosecurity messages. Consequently, provision of information is timely, and although from an IT system, its *Messenger* is trustworthy, *Defaults* are built into the supportive system, *Salience* is elicited and farmer *Commitment* is encouraged (and required), throughout the programme.

Of the strategies that aimed to encourage *adoption of biosecurity practices* in general, not linked to specific disease control activities, none were considered to address three or more attributes of the EAST framework; they are thus not elaborated upon further.

DISCUSSION

This study confirmed that behavioral influence strategies have been commonly considered in implementation of animal health surveillance and disease control activities in many European countries, and that these strategies are applied both by authorities and industry-based animal health services. In general, choice of strategy is based on professional empirical understanding of requirements to achieve the anticipated outcomes, rather than systematic use of methods from behavioral sciences/psychology. However, almost half of strategies described here, were designed in manners that fulfilled existing recommendations for successful uptake of policies (Service et al., 2015), including multiple triggers of psychological mechanisms that support conscious or intuitive actions (Webb & Sheeran, 2006, Dolan et al., 2012).

Although the actual context in which information strategies were delivered differed, there are some common themes with respect to how content was framed and context or environment was modified. For example, irrespective of the desired behavior, it was common to use, appoint or engage a trustworthy *Messenger* to deliver information, and ensure that context was empowering from a social perspective, triggering mechanisms such as *Norms, Commitment* and *Ego* or generating *Salience* or a *Priming* effect by campaign-like implementation strategies.

Some MINDSPACE mechanisms, such as *Salience* and *Affect*, have less predictable outcomes as they are highly individual and may involve emotions and professional decisions which are positively or negatively regarded. Accordingly, behavioural influence strategies can also backfire (Cialdini, 2003; Sunstein, 2017), if perceived as condescending or activating psychological mechanisms of trust and commitment without solid justification. For example, over-communicating consequences of *E. multilocularis* in terms of public health impact, triggering a sense of commitment and societal responsibility, was reported as a potential reason for hunters in SE disengaging in surveillance after realising the diseases' rarity. In NL, the use of inappropriate *Messengers* (authority representatives informing poultry farmers about surveillance without having an understanding of the production system) resulted in farmer disengagement due to lack of trust, with subsequent negative impacts on reporting of disease events (data not shown).

Only ten of 51 strategies were described as supporting desired behaviors by making them *Easy* to carry out. This indicates an opportunity for improvement in strategy design by, for example, considering defaults, reducing the 'hassle factor' of carrying out a desired behavior or simplifying messages (Bettinger et al., 2009; Service, 2015). In particular, it could be important in maintaining compliance with, or acceptance of an intervention (e.g. a control scheme) (Sunstein, 2017), as exemplified by text message reminders for culling of persistently BVDV infected animals in IE. In general, smartphone solutions are well suited as nudging tools

due to their potential timeliness and availability, making it easier for individuals to maintain desired behaviours (Fishbach and Hofmann, 2015).

Notably, the impact of strategies has not been assessed in this study; this is for later work, either using randomised controlled trials (Haynes et al., 2012), or possibly retrospective cohort studies. Despite this, the study participants shared their views about the effectiveness of strategies, and why. Furthermore, more solid evidence building is underway; one interviewee reported Danish farms where solutions based on nudge theory are facilitating good biosecurity behaviour and reducing risk of work related injuries (SEGES, 2017). However, until now there has been little implementation research related to behavioural influence strategies in animal health surveillance and disease control. The frameworks presented here could serve as guidance in design of future interventions and surveillance activities. Further, incorporation of assessments of the effectiveness of behavioural influence strategies into evaluations of animal health interventions would be beneficial for further development of evidence-based animal health policies.

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MATERNAL VACCINATION AS A *SALMONELLA* TYPHIMURIUM REDUCTION STRATEGY ON PIG FARMS

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SUMMARY

A longitudinal field study was carried out to investigate the efficacy of vaccination with an inactivated *S.* Typhimurium vaccine in breeding sows to reduce the prevalence of *Salmonella.* Sows in eight commercial farrow-to-finish herds were vaccinated and results were compared against eight comparable control farms. At the final visit (~14 months after the start of vaccination), when all finishing stock had been born to vaccinated sows, both faecal shedding and environmental prevalence of *Salmonella* had substantially declined on the majority of vaccinated farms in comparison to the controls. A higher proportion of vaccinated farms resolved clinical salmonellosis than controls. The results suggest that maternal vaccination is a suitable component of a *Salmonella* Typhimurium reduction strategy in farrow-to-finish pig herds.

INTRODUCTION

The 2015 European summary report on foodborne outbreaks reported that *Salmonella* was responsible for the majority (21.8%) of foodborne outbreaks in humans, in the European Union (EU) (EFSA 2016). Pork is considered, after eggs, the major source of infection in humans in the EU, with *S.* Typhimurium, including monophasic strains (*S.* 1,4,[5],12:i- and *S.* 1,4,12:i-) being frequently implicated (Andres and Davies 2015; Davies *et al*. 2016). Nonetheless, within the EU, there is no mandatory programme for the control of *Salmonella* at pork primary production level.

The persistent and predominantly asymptomatic nature of porcine *Salmonella* infection and the organism's ability to colonize other animal species, such as rodents and wild birds on farms, and to survive in the environment means that effective control requires multiple measures (Wales and Davies 2017). In summary, control measures against *Salmonella* infection can be divided into five broad interventions: biosecurity, feed formulation, acidification of feed or water, manipulation of gut microbiota, and vaccination (Andres and Davies 2015; Wilhelm *et al*. 2016). Wilhelm *et al*. (2016) suggests that biosecurity and vaccination seem to be the

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intervention categories showing the greatest potential to minimise *Salmonella* on an infected farm, but only culling of infected pigs can totally eliminate infection.

It is generally accepted that vaccination can play a role in reducing the prevalence of *Salmonella* in pigs and could become an adjunct to other on-farm control measures (Denagamage et al. 2007). Relatively few vaccination studies involving *Salmonella* have been undertaken under field conditions on pig farms and most of these have been conducted with small numbers of animals (Schwarz et al. 2011; Arguello et al. 2013; De Ridder et al. 2014; Ruggeri et al. 2015; Davies et al. 2016).

In the present study, a long-term longitudinal field investigation was developed which aimed to evaluate the efficacy of vaccination with a live attenuated *S.* Typhimurium vaccine administered to all breeding sows present in the herd, as a strategy to reduce the prevalence of *Salmonella* infection on farms. For further details of the study please see Smith *et al* (2017).

MATERIALS AND METHODS

Farms

A total of 35 farms were invited to participate in the study in order to identify 16 eligible and willing participants. Farms were selected based on the following inclusion criteria: (i) indoor breeder-finisher enterprise, (ii) herd size of 100-600 sows, (iii) recent occurrence of *S.* Typhimurium (ST) or monophasic variant (mST), (iv) presence of ST or mST in finishing pigs, (v) farmer willing to be involved for the entire study period, and (vi) sows free of significant clinical disease which could affect the efficacy of the vaccine.

Sampling visits and vaccination schedule

Farms were randomised into vaccinated (n=8) and non-vaccinated groups (n=8, recorded as control farms from this point on). Farms were followed for approximately 69 weeks after the start of the trial, with four sampling visits. Sows were vaccinated with a live attenuated vaccine by subcutaneous injection (Salmoporc STM, IDT Biologika GmbH, Dessau-Rosslau, Germany). Vaccine was administered to pre-partum sows (6 weeks and 3 weeks ante-partum) with a single booster dose at three weeks before each subsequent farrowing. The first dose was given to the first batch of sows in week 1 and the second dose in week 4. The piglets (progeny) from the first batch of vaccinated sows were estimated to go to slaughter during week 33. The last batch of sows was vaccinated in weeks 23 and 26 and farrowed in week 29, with their progeny going to slaughter in week 55. Sampling visits took place prior to vaccination (week 0); at a point when half of the progeny on the farm were estimated to originate from vaccinated sows (week~21); when all the finishers on the farm were from vaccinated sows (week~55); and there was a final "follow-up" sampling visit, which took place three to four months later (week~69). Sows were observed closely for any signs of ill health after vaccination.

Sampling and *Salmonella* detection

A target of sixty individual floor faecal samples were collected at each visit from each of the following pig stages: weaners, growers, and finishers, providing a 95% probability of detection assuming a 5% prevalence and 100% sensitivity of detection (EpiTools epidemiological calculators, Ausvet Pty Ltd). Faeces were collected in sterile stool sample tubes using an integral spoon. In addition, pooled pen faecal samples (one or two pools per pen

according to the number of pigs in the pen) were taken from the following pig stages: gestation sows, farrowing, weaners, growers, finishers and a representative group of gilts and boars. For each pig stage, up to a maximum of 20 samples were collected per building and 60 per pig stage to ensure effective detection of *Salmonella* prevalence and diversity of serovars across the farm. Pooled samples were collected using a sterile gauze swab held with a clean disposable glove for each sample. In addition, wildlife faeces and environmental samples were collected.

Solid and semi-solid material (up to 25 grams (g)) was collected using sterile gauze swabs which were wiped in a zigzag path over a two-metre length. Environmental samples were taken with gauze swabs that had been pre-autoclaved in buffered peptone water (BPW), and 50 millilitres (ml) was collected from water sources.

All pooled faecal samples (approximately 25g) and swabs were pre-enriched in 225ml BPW at 37 degrees centigrade (°C) for 18 hours (h) followed by enrichment in Modified Semi-Solid Rappaport-Vassiliadis medium (MRSV) for 24h or 48h at 41.5^{o}C then plated on Rambach agar which was incubated for 24h at 37^{o}C (Martelli et al. 2014). Sub-samples (2g) of individual pig faeces were pre-enriched in 20ml BPW and cultured as above.

A selection (all isolates from pooled samples and any individual sample that was cultured semi-quantitatively) of *Salmonella* isolates were fully sero- and phage-typed in the Animal and Plant Health Agency (APHA) *Salmonella* reference laboratory using standard methodology to define *S.* Typhimurium or monophasic variants (ST/mST)(Jones, McLaren and Wray 2000).

Details were collected on whether the farm's veterinary practitioner had identified clinical salmonellosis in the pigs since the last sampling visit. This was used to assess whether vaccination may have reduced the number of farms showing clinical symptoms in comparison to control farms.

Statistical analyses

For analysis of the effect of vaccination, a mixed-effects logistic regression model was used to examine the association between time from the start of vaccination (represented by visit number, with the first visit being before the introduction of vaccination) and the odds of a sample being *Salmonella*-positive, the hypothesis being that vaccination would progressively reduce the odds of a sample being positive over time. The *a priori* variables were pig stage from which the sample was collected (named pig type), sample type (individual or pooled), and season (winter (Dec-Feb), spring (Mar-May), summer (Jun-Aug) and autumn (Sep-Nov)). Farm study identifier was added as a random effect to account for non-independence of sample results from the same farm. The use of farm and group random effects were tested, but the addition of group did not significantly improve the fit of the model (Likelihood Ratio test). An interaction term, including visit number and experimental group, was added to allow for different effects of the vaccine over visits on the different farms. Two outcomes were tested in the model: *Salmonella*-positive or ST/mST-positive. All analyses were performed in Stata 12 (StataCorp, 2011. Stata Statistical Software: Release 12. College Station, TX: Stata-Corp LP). A p-value of less than 0.05 was considered an indication of a statistically significant difference.

RESULTS

Data from 16 farms are presented in this study. Of the eight farms in the vaccinated group, five employed a weekly batch sow management system and the other three farms employed

two, three and four week batch systems, respectively. In the control group, seven farms used a weekly batch management system and one farm employed a three-week batch system. The mean number of sows and gilts per herd was 321 (range 150 to 550) for vaccinated farms and 406 (range 150 to 750) for control farms. Clinical problems (diarrhoea, septicaemia, ill-thrift and increased mortality) associated with *Salmonella* infections were reported from six vaccinated and three control farms, respectively. ST/mST serovars had been detected in weaned pigs on all farms before the start of the trial.

Bacteriological results

A total of 22,246 samples (9,747 pooled faecal samples, 10,905 individual faecal samples and 1,594 environmental samples) were collected between April 2014 and May 2016, with an intense level of sampling per visit (mean of 374 samples collected in each visit). ST/mST were the predominant serovars detected, consisting of more than 90% of isolates. The initial visit (visit 1) results demonstrated a similar high prevalence of *Salmonella* from faecal samples in both vaccinated and control groups; 30.8% vs 36.2% of pooled samples, 19.1% vs 21.9% of individual samples, and 34.6% vs 53.0% of environmental samples, for vaccinated versus control groups, respectively. The proportion of *Salmonella*-positive samples ranged from 3.7% to 62.2% on vaccinated farms and from 11.5% to 67.0% on control farms in pooled samples.

Figure 1 summarizes the effect of sow vaccination on the *Salmonella* sample prevalence of pigs for all rearing stages. Weaners and finishers born from vaccinated sows showed significantly reduced *Salmonella* sample positivity from the first to the last visit ($p=0.006$ and $p<0.001$, respectively). Samples from growers born from vaccinated sows also showed reduced *Salmonella* prevalence, although the difference was only approaching significance ($p=0.057$).

The effect of vaccination was not consistent on all farms; on one farm, prevalence increased at visit 2 and this rise was sustained up to the final visit for both pooled (3.7%, 35.8%, 29.5% and 38.5% for visits 1, 2, 3 and 4, respectively) and individual samples (0.0%, 16.2%, 26.9% and 23.3% for visits 1, 2, 3 and 4, respectively). Another vaccinated farm showed only a slight reduction after vaccination, with a similar sample prevalence observed at visits 2 and 3 to that at the beginning of the experiment (20.1%, 8.6%, 17.6% and 19.3% of pooled samples for visits 1, 2, 3 and 4, respectively, and 16.5%, 12.2%, 18.9% and 12.4% of individual samples for visit 1, 2, 3 and 4, respectively). However, it should also be noted that although two vaccinated farms retained a ST/mST prevalence (in pooled samples) of over 20% at the final visit, no vaccinated farm had a prevalence of over 20% using individual samples. Details of farm structure and management (such as flooring type and use of acidified feed) were investigated, but no apparent difference was detected that could explain the difference in treatment effect.

Findings from the mixed-effects models are summarised in Table 1. There was a significantly decreased odds ratio of *Salmonella*-positive (Odds Ratio (OR) = 0.726, $p<0.001$) and ST/mST-positive samples (OR=0.706, $p<0.001$) for vaccinated farms in comparison to control farms. In addition, samples collected at visit 2 were at significantly lower odds for both outcomes than at the first visit. Examining the interaction between the experimental groups and visit number showed that there was a significantly decreased odds ratio of *Salmonella*-positive (OR=0.512, $p<0.001$) or ST/mST-positive (OR=0.613, $p<0.001$) samples at visit 4 for vaccinated farms when compared to control farms at visit 4. The analysis of the sample type on all the farms revealed a significant increased odds of isolation in pooled samples of *Salmonella* and ST/mST, when compared with individual samples (OR=2.697, $p<0.001$ and OR=2.558, $p<0.001$, respectively).

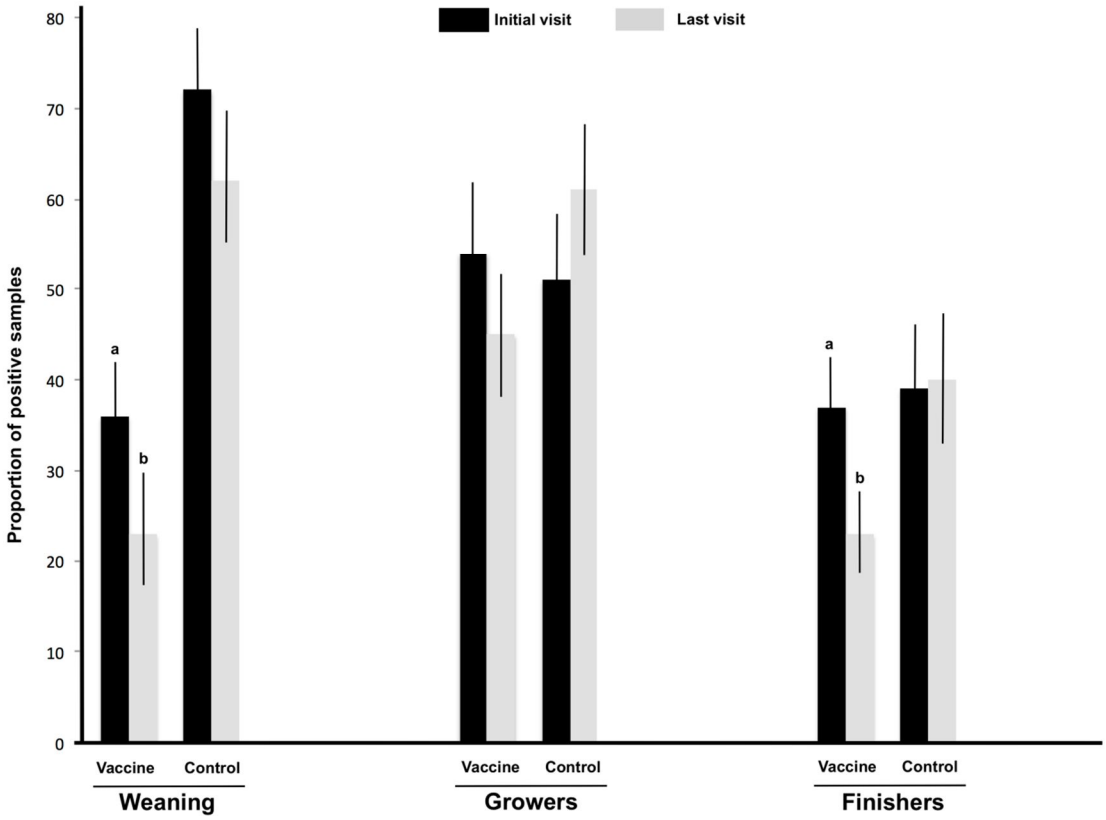

Fig. 1 Results of shedding *Salmonella* in faecal pooled samples of piglets born to vaccinated and control sows at weaner, grower and finisher rearing states. Data are expressed as mean ± standard error. a,b pairs, indicate results, within rearing states, which are significantly different from each other ($p<0.05$).

There was a significantly increased odds of isolation in summer of *Salmonella* (OR = 1.214, p=0.004) or ST/mST (OR=1.198, p=0.013) and an increase in spring and autumn for ST/mST-positive (OR=1.119, p=0.025 and OR=1.130, p=0.047, respectively) when compared with winter. Finally, the model showed significantly increased odds ($p<0.001$) of *Salmonella*-positive and ST/mST-positive samples for all pig group types (except boars) and significantly reduced odds of both outcomes for farrowing groups, when compared against the gestation group, with weaners presenting with the highest odds of detection.

By the final visit, only one of the original six vaccinated farms (17%) reporting clinical symptoms were still presenting with clinical cases, in comparison with one of three (33%) controls.

DISCUSSION

This study is the first extensive controlled investigation to demonstrate that a strategy of maternal vaccination against *Salmonella* Typhimurium reduces, by a substantial proportion of treated farms, both faecal and environmental prevalence of *Salmonella* in farrow-to-finish pig herds, especially for serovars *S.* Typhimurium and its monophasic variants. Nevertheless, according to previous work, although a beneficial association between vaccination and *Salmonella* reduction was observed, vaccination strategies alone are not sufficient to eliminate

Table 1. Mixed-effects logistic model summarising the association between vaccination and the presence of *Salmonella* and *Salmonella* Typhimurium and its monophasic variants (ST/mST), whilst accounting for *a priori* variables, from a controlled trial of 16 pig farms (and n=22,246 samples).

		***Salmonella*-positive**		**ST/mST-positive**	
Variable	**Level**	**Odds ratio**	***P*-value**	**Odds ratio**	***P*-value**
Farm type	Control	Ref.			
	Vaccinated	0.726	<0.001	0.706	<0.001
Visit x farm type	1 x Farm type	Ref.			
	2 x Farm type	1.070	0.492	1.311	0.008
	3 x Farm type	1.028	0.775	1.043	0.667
	4 x Farm type	0.512	<0.001	0.613	<0.001
Sample type	Individual	Ref.			
	Pooled	2.697	<0.001	2.558	<0.001
Season	Winter	Ref.			
	Spring	1.090	0.070	1.119	0.025
	Summer	1.214	0.004	1.198	0.013
	Autumn	1.069	0.268	1.130	0.047
Pig type	Gestation	Ref.			
	Boars	1.496	0.564	1.842	0.381
	Farrowing	0.559	<0.001	0.610	<0.001
	Weaners	6.292	<0.001	6.995	<0.001
	Growers	5.349	<0.001	6.119	<0.001
	Finishers	3.261	<0.001	3.732	<0.001
	Gilts	1.733	<0.001	2.069	<0.001
	Environmental	4.252	<0.001	4.987	<0.001
	Dry sows	2.269	<0.001	3.061	<0.001
	Mixed	3.252	<0.001	3.640	<0.001
Visit	1	Ref.			
	2	0.783	0.001	0.721	<0.001
	3	0.890	0.086	0.934	0.326
	4	1.095	0.193	1.045	0.539

infection that is present on pig farms and all vaccines aimed at intestinal bacteria should preferably be applied to uninfected animals on a preventative basis rather than in the face of infection (Wales et al., 2011; Soumpasis et al., 2012). The persistent and frequently asymptomatic nature of porcine *Salmonella* infection and the organism's ability to colonize other animal species and survive in the environment, means that effective control of subclinical *Salmonella* infection generally requires multiple approaches applied simultaneously (Wilhelm et al., 2016; Wales & Davies 2017), although clinical salmonellosis can usually be markedly improved by vaccination alone, as demonstrated in the current study. Vaccination may assist in the protection of animal health, reduction of antibiotic usage, enhancement of food safety as well as reduction of economic losses and environmental contamination associated with faecal waste, run-off and transmission of *Salmonella* to other food animal species, such as poultry, by wildlife vectors (Andrés-Barranco et al., 2014; Bearson et al., 2016).

Vaccination is the second most frequently studied on-farm intervention measure for *Salmonella* control (Wilhelm et al., 2016). However, longitudinal field studies (such as the present one) examining natural infections are uncommon in pig trials (Davies et al., 2016; Wilhelm et al. 2016). This study was novel in that the trial was run under controlled field conditions, using a large number of animals, and focusing on farms with an existing *Salmonella* problem. Although direct comparison with previous studies must be applied carefully owing to inherent experimental differences (Ruggeri et al., 2015; Davies et al., 2016), these results confirm that vaccination of sows can reduce the prevalence of *Salmonella* in farrow-to-finish pig herds. In addition, these results highlight an important reduction in contamination of the farm environment.

There are a number of strategies that may be used when implementing vaccination of pigs against *Salmonella* (Wales & Davies 2017), for example: immunisation of sows to protect their offspring (Roesler et al., 2006; Ruggeri et al., 2015; Davies et al., 2016); or vaccination early in the pig's life (Hur & Lee 2010; Schwarz et al., 2011; De Ridder et al., 2014; Ruggeri et al., 2015), during suckling (Hur et al., 2001), after weaning (Berends et al., 1996; Kranker et al., 2003; Merialdi et al., 2008) or during fattening (Arguello et al., 2013). It has been previously reported that when sows were vaccinated, the prevalence of *Salmonella* shedders, as well as of seropositive pigs within progeny, was reduced and it was suggested that vaccination of breeding sows could be an easy-to-apply and economic way to reduce *Salmonella* transmission to progeny and enhance maternal immunity. Vaccinating the piglets would have required a much larger number of doses and a greater labour cost in dosing litters and weaners. Other studies have suggested that additional vaccination of suckling piglets and weaners would provide further benefits, but this is less easy and economical to carry out in many farming systems (Roesler et al., 2006; Andres & Davies 2015; Ruggeri et al., 2015). Vaccination protection of sows is particularly relevant in farrow-to-finish pig herds where breeders and finishing pigs are housed in the same environment and weaned pigs present a continuous source of environmental contamination with ST or mST (Lurette et al., 2009). The carriage of *Salmonella* by piglets is readily demonstrated from farrowing accommodation onwards (Jones et al., 2000). According to Kranker et al., (2003), *Salmonella* is predominant in weaners, growers, and finishers. Nevertheless, once all sows were vaccinated, a reduction in *Salmonella* prevalence was observed in all stages of pig production, and mainly in finishers, hence reducing the total *Salmonella* burden before slaughter, at the beginning of the pork-based food chain. The reduction in shedding by growing pigs in farrow-to-finish pig herds is also consistent with enhanced passive immunity, clearance of infection and reduced carriage of infection by weaners, eventually maturing into growers and finishers (Davies et al. 2016). Although previous findings have shown that pigs born from vaccinated sows have reduced *Salmonella*

in faecal shedding (Roesler et al., 2006; Matiasovic et al., 2013), a reduction in environmental contamination and re-cycling of infection is also important (Davies et al., 2016). Collectively, this data suggest that maternal vaccination can significantly reduce carriage of *Salmonella* in the progeny of vaccinated pigs, as well as environmental contamination.

It is known that live attenuated *Salmonella* Typhimurium vaccines can help prevent clinical salmonellosis, reducing tissue colonisation and faecal shedding (Roesler et al., 2004; Gradassi et al., 2013). These results show that a larger proportion of vaccinated farms had ceased to show clinical signs in comparison to controls. Vaccination can thus provide a potential economic and welfare benefit to pig production.

Although other serovars were isolated, ST and mST accounted for ~90% of all isolates. As ST and mST are serovars posing the largest risk to humans within the United Kingdom (UK) pig reservoir, it is important that these are controlled using vaccination, especially when combined with good biosecurity, management, and farm hygiene practices (Andres & Davies 2015).

These results provide evidence that maternal vaccination in farrow-to-finish pig herds is a suitable ST/mST reduction strategy and helps to control clinical salmonellosis. *Salmonella* vaccines therefore have the potential to reduce prevalence of this infection in pigs, which would likely result in a reduction of human cases attributed to pork. However, more research is required to quantify impacts throughout the pig meat production chain. For further details of the study please see Smith et al., (2017).

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SMART DATA FOR VETERINARY EPIDEMIOLOGY: COMPARING VARIOUS MACHINE LEARNING ALGORITHMS FOR DETECTION OF LAMENESS IN SHEEP

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SUMMARY

There has been an increase in systems for precision livestock monitoring in the past decade. Automated behavioural classification and identification through sensors has the potential to improve health and welfare of the animals as these behavioural monitoring systems can be used to detect changes in behaviour that are associated with disease. However, these monitoring systems generate huge amounts of information that require processing and subsequent communication. Therefore, to eventually deliver a complete precision livestock monitoring framework in veterinary epidemiology that can efficiently monitor in real-time and for long durations, factors that have an impact on processing, communication and power consumption have to be evaluated. Using sheep behaviour and lameness as an example, some of these factors are explored including sampling frequency, sensor position, and window sizes on the performance of automated classification. Associated challenges of such 'big data' techniques are also discussed.

INTRODUCTION

Discussion on the use of 'big data' techniques is rapidly growing. Advances in animal-borne technologies such as global positioning system (GPS) trackers, location sensors, proximity loggers, accelerometers, gyroscopes and magnetometers have allowed researchers to automatically detect and classify different relevant behaviours in wild and farm animals (Valleta et al., 2017; Neethirajan et al., 2017) by collecting, assimilating and analysing the information they provide using various machine learning approaches. These technologies facilitate analysis of animal behaviour patterns to inform farm decision-making, hence improving animal welfare and health. In particular, for example in sheep, an accurate and precise monitoring system can help to detect behavioural changes (i.e. abnormal postures) that are linked to health and welfare status (i.e. foot lesions) (Kaler et al., 2009). Changes in sheep normal stance or gait that have been associated with lameness can be used to develop an automatic lameness detection system and help to treat individuals earlier, to reduce disease spread.

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Sensor technology encompassing accelerometer and gyroscopes has been widely used by researchers to classify different behaviours in wild and farm animals (Martiskainen et al., 2009; Vázquez-Diosdado et al., 2015; Moreu et al., 2009; Alveranga et al., 2009). Advances in these technologies have made it possible for sensors to process and transfer an increasing amount of data. Such large data resources are typically processed using various machine learning algorithms such as: support vector machines, random forest, etc. However, a key question remains over how to convert such 'big data' (given its volume, velocity and variety) into 'smart data'.

One key factor in this conversion is data processing. Processing can be undertaken either by using a cloud-based system (requiring real-time streaming of data) or by using embedded based architecture (processing performed on the device). Each of these architectural systems has its own limitations: cloud-based systems have a high power drain and reduced life battery while embedded systems are limited by the processing power and memory on each device (Jukan et al., 2017). Therefore, an architectural solution to monitor animals in real-time and for long durations requires optimisation of the parameters on such devices. Some of the factors that considerably affect energy transmission, bandwidth and storage capacity include: sampling rate, window sizes and sensor position. Although there is a vast amount of literature describing the systematic evaluation of human 'big data' processing (Janidarmian et al., 2017), there is a huge gap for livestock behaviour monitoring (Jukan et al., 2017; Neethirajan et al., 2017; Valleta et al., 2017).

This study aimed to explore the development of a device to be used for real-time monitoring, behaviour classification (lying, standing and walking) and early detection of lameness in sheep; examining the challenges of converting 'big data' to 'smart data'. The effects on performance of automated classification of sheep behaviour using three different sampling frequencies (8 hertz (Hz), 16Hz and 32Hz), three window sizes (3s, 5s, and 7s) and two sensors positions (ear and collar), using tri-axial accelerometer and tri-axial gyroscope data, are presented. Finally, initial results are summarise describing classification of lameness in sheep using such a device.

MATERIALS AND METHODS

Study site and animals

For this study, a total of 19 sheep were selected via stratified random sampling from a flock of 140 animals at the University of Nottingham. Data was collected, over a total of 10 days, in 2016 and 2017. Sheep had a range of body condition scores (2.5 to 4), and were of different ages (18 months–4 years) and breeds (Texel cross, Suffolk cross and Mule).

Data collection

Sensor data were collected using a custom-made wearable device based on the Intel® Quark™ SE microcontroller C1000. The device encompassed flash memory, a low power wide area radio module and Bosch integrated ±8 gram low power inertial measurement unit (IMU), featuring a 16 bit tri-axial gyroscope and 16 bit tri-axial accelerometer. Sensors were attached to the sheep at two different locations: a) using the existing electronic ear tag identification and b) to a neck collar. In this study, the device sensors were set to collect data at sampling frequencies of 8Hz, 16Hz and 32Hz per axis, at the beginning of each day.

Behavioural observations

Sheep behavioural activities were recorded using a handheld Panasonic HC-V380 video camera and tripod, and were time-stamped. Video recordings were set in an MP4 50M format with 1080 pixel (1920X1080) quality. Video footage was recorded each day for approximately 4 hours, and the start and end times were annotated. Processing of the time-stamped video recording was performed using the Noldus Observer XT11 (Noldus) (www.noldus.com). Using this software, video records were coded using a predefined set of behaviours. Three different behaviours were investigated within this study using an ethogram: walking (sheep moving for two seconds or more), standing (sheep standing with four legs, with or without jaw movement) and lying (sheep lying on the ground in sternal or lateral recumbence). Lameness was scored according to a scale previously validated by Kaler et al. (2009), with non-lame scored as zero and one, and lame as two or above.

Data processing

Sensor data (accelerometer and gyroscope) was processed using custom written files in Python 3.5 by first aligning with behavioural information from the video recordings. Secondly, sensor data was partitioned using equal windows of three, five and seven seconds, with 50% overlap between two consecutive windows. Finally, a behavioural label was assigned to each window.

Feature characteristics were extracted from the magnitude of the acceleration and the gyroscope. The magnitude of the acceleration, where A_x, A_y, A_z represents the acceleration in the x, y and z axes, respectively, and magnitude of the gyroscope defined in a similar manner, can be computed, see Eq. (1).

$$\overline{\boldsymbol{A}} = \sqrt{A_x^2 + A_y^2 + A_z^2} \tag{1}$$

From the magnitude of accelerometer and gyroscope and the rate of change of their magnitude, eleven feature characteristics were extracted based on previous work (Walton et al., 2018), resulting in a total of 44 feature characteristics.

Behaviour Classification algorithm: For the evaluation of sensor position, sampling frequency and window size, only one algorithm was employed. A random forest learning algorithm using Microsoft Azure Learning Studio software was chosen, based on initial analysis. Data was partitioned into a training (70%) and test set (30%) using random stratification to ensure correct ratios between the different activities (Kpedekpo et al., 1973). More detailed information on the processing and parameter settings for the classification algorithm can be found in Walton et al. (2018).

Performance of the classification: The performance of the classifiers was computed using different metrics such as accuracy, precision, recall, F-score and specificity. Additionally, Cohen's weighted Kappa was calculated to assess the level of agreement between the two different sensor positions. The effect of energy consumption when using a three, five and seven second window size, on the device (Intel®), was also computed.

Lameness classification algorithm

Lameness in sheep is typically identified through visual inspection of animals, and locomotion scoring, which happens whilst walking. This provides the possibility of using

sensor information for walking behaviour to develop a second classifier for the identification of lameness. Therefore, a detection algorithm for the identification and classification of lameness was designed following a two-stage process, where in the first stage, discrimination of behaviour occurs, and in the second stage, detection of lame walking happens.

Lameness classification was explored using several different classification algorithms including: random forest neural network, averaged perceptron, logistic regression, support vector machines and Bayesian point machines. Only data with fixed sampling rates and window size was used for this. Every algorithm was trained using up to ten of the most informative features according to their mutual information score and utilising a 10-fold cross validation (Rodríguez et al., 2010). The lameness classification results on a window basis were used to define the ratio of windows, where a sheep was classified as lame over the total number of windows in a period of time, which then provided a sheep's individual level classification.

RESULTS

Table 1 describes a comparison of accuracy of the automated classification across all the window sizes and sampling frequencies, for both ear and collar mounted sensors.

Table 1. Overall accuracy of ear and collar data across all frequencies and window sizes.

Sensor position	Sampling frequency	Window size (seconds)		
		3	5	7
Ear	8	89	91	93
	16	89	92	92
	32	94	95	95
Collar	8	89	90	92
	16	90	93	93
	32	95	95	95

The highest overall accuracy was 95%, which was obtained for ear data at 32Hz with five and seven second windows, and for collar data at 32Hz for all windows. The lowest overall accuracy was 89%, obtained for ear data at sampling frequencies of 8Hz and 16Hz with a three second window size, and for collar data at a sampling frequency of 8Hz with a three second window size. Overall, the accuracies observed were worst for three second windows and best for seven second windows.

Results of the Cohen's weighted Kappa measures show that except for a combination of 8Hz and three seconds, all Kappa values were above 0.80, representing a good agreement. When comparing across different sampling frequencies, the worst Kappa values were obtained at 8Hz and the best at 32Hz. When comparing across the different window sizes, the best results were obtained for seven seconds.

Performance for specific activities

Walking: Ear and collar data produced very similar classification results for walking (precision of 89.33% and recall of 87.77% on average for ear; precision of 88.33% and recall of 88.66% on average for collar). The lowest performance values were obtained on a three second window and 8Hz sampling frequency for ear and collar data with values of 80% recall.

Standing: Performance on the classification of standing were very similar to walking as performance values ranged between 81% and 96% for ear data and 81% and 97% for collar data. Similarly to walking, the highest performance values were obtained for specificity, with values ranging between 92% and 96% for ear data and 93% and 96% for collar data. The classification performance of standing for ear data (precision of 89.22% and recall of 88.55%, on average) was similar to collar data (precision of 89.55% and recall of 88.66%, on average).

Lying: Lying had the highest performance values with above 93% for precision, recall, F-score and specificity in collar data. Precision (positive predictive) values were 95.21% on average, recall values were 95.99% on average in both ear and collar. For the three different behaviours, lying had the highest overall performance for all window sizes and sampling frequencies.

The classification performance for standing and walking behaviours improved with increasing sampling frequency and window size. Overall the best results were observed when using a 32Hz sampling frequency and seven second window with an average precision of 95% and average recall of 94.33% across the three behaviours.

Energy consumption

Energy consumption benefits of using a seven compared to five or three second window are shown in Table 2. These benefits include a reduction in the energy required to acquire samples and a decrease in energy used to write to flash.

Table 2. Energy consumption parameters for three different window sizes assuming a fixed sampling rate.

Measure	Window size (seconds)		
	3	5	7
µAh energy (sample acquisition/hr)	333	200	143
µAh energy (writes to flash)	10	6	4

Lameness classification

Table 3 summarises the overall accuracies obtained using the different algorithms. The highest overall accuracy was for random forest (68.6%), while the lowest was for linear regression and averaged perceptron (59.3% for both). The highest precision values were also for random forest (67.8%), and the lowest for support vector machine (59.5%). The highest recall values were for random forest (78.3%) and the lowest were for linear regression (68.7%).

Table 3. Accuracy of lameness classification.

Method	Accuracy	Precision	Recall
Random Forest	68.6	67.8	78.3
Neural Network	61.4	60.3	69.2
Bayes Point Machines	59.2	60.3	69.0
Support Vector Machine	58.1	59.5	71.3
Linear Regression	59.3	60.5	68.7
Averaged Perceptron	59.3	60.6	69.3

The results of the lameness classification at a window level were relatively low (59.3% to 68.9% accuracy), hence the ratio of windows where an individual was classified as lame was used to discriminate between two different classes (non-lame containing LMS 0 and 1) and lame (containing LMS 2 and 3). Figure 1 illustrates the results for individual classification using the proportion of windows with the binary classification (lame vs non-lame). These suggest that using the ratio of windows, it should very easy to define a threshold to discriminate between lame and non-lame sheep.

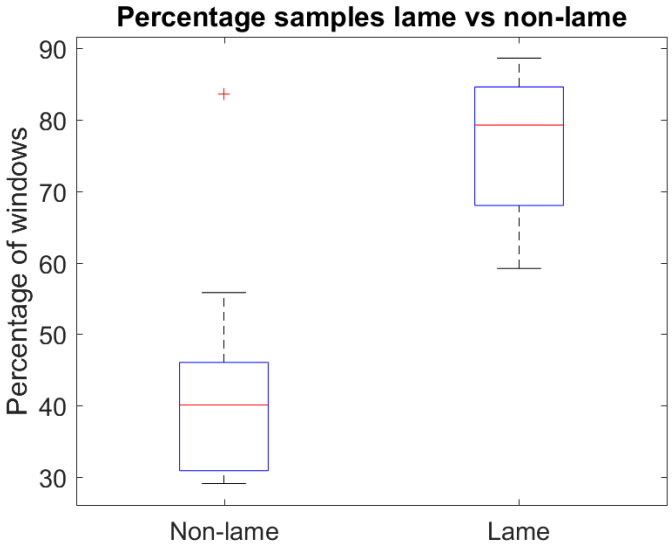

Fig. 1 Boxplot of the ratio of windows where a sheep was classified as non-lame or lame.

DISCUSSION

This study demonstrates that sampling frequency, position of the sensor window size and choice of algorithm impact the predictive performance of automated classification of sheep behaviour and lameness. These parameters are important in decision making for converting 'big data' generated via sensors to 'smart data' that could be used for real-time monitoring. The implications of these points are discussed.

When comparing the performance of classification of sheep behaviour between ear mounted and collar mounted sensors, very similar results were obtained (0.44% average difference in accuracy, 0.14% in precision, 0.41% in recall). Differences in performances might be due to relatively higher noise in the ear data, resulting from a high degree of freedom in the movement of the sensor. With such small differences between ear and collar, the selection of sensor position relies on its practicality, with an advantage for the ear sensor in that it could possibly be integrated into the current electronic identifier.

The performance of automated classification was best for all three sheep behaviours when using a seven second window, and in general, classification increased with increasing window size. This is in agreement with a recent study by Alvarenga et al. (2016) showing an increase in accuracy with an increasing window size for various sheep behaviours. Window sizes can affect the performance of automated classification in different ways, with longer windows more suitable for complex behaviours as they contain more information, resulting in better accuracy. For example, this was observed in this study where performance of standing and walking improved with window size, while lying was very similar across all window sizes. Not only does window size impact performance, but also energy consumption, thus it is a key

consideration for real-time monitoring. In this study, there was saving of up to 58% of energy when changing from a three compared to seven second window.

The performance of automated classification also depends on the type of behaviour being classified. Misclassification mainly occurs between standing and walking, probably due to standing also containing grazing behaviour, resulting in accelerations close to those obtained when walking. Lying was the behaviour with the highest performance values, possibly explained by significantly less movement in this behaviour compared to standing or walking. Despite the lameness algorithm having an overall accuracy of around 70% at window level, the aggregate ratio of windows was able to classify lameness correctly at individual sheep level. This suggests that for certain classification problems, aggregate measures can be implemented into the logics of the device to help achieve high accuracy for real-time monitoring solutions, despite relatively lower accuracy results for shorter windows.

There are no algorithms available to aid classification of sheep lameness, and studies on cattle lameness have generally used very high sampling rates. For example, Vázquez-Diosdado et al. used a 50Hz sampling rate while Alvarenga et al. (2016) used up to 25Hz sampling frequencies. The results from the current study indicate that despite some gain in overall accuracy at the highest sampling rate, penalties in the energy used for processing may hamper utilisation of high sampling rates in real-time lameness detection scenarios (Kahn et al., 2016).

Another factor impacting automated classification of both behaviours and lameness is selection of the machine learning algorithm. Such selection not only impacts classification performance, but also the computation cost of the behavioural monitoring system. In comparison to previously used classification algorithms in livestock (Vázquez-Diosdado et al. (2015) using decision trees and Hidden Markov models, and Martiskainen et al. (2009) using support vector machines), random forest has the advantage of improving the over-fit of decision trees, while maintaining a low computation cost, compared to support vector machines or other algorithms, or Hidden Markov models (Valleta et al., 2017; Neethirajan et al., 2017). Moreover, the random forest algorithm obtained the highest performance in automated classification of lameness, making it suitable for use in both discrimination of behaviour and detection of lameness without the need to implement an additional algorithm. Therefore, a random forest algorithm is an ideal candidate for implementation on an embedded real-time device producing high levels of accuracies.

Whilst the algorithm (with 10-fold cross validation) in the current study shows high classification accuracy for behaviour and lameness, further validation of its performance is being undertaken in a larger trial. This will explore the impact of various factors such as sheep breed, age, time of year and other variables on the accuracy of algorithms.

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AVIAN INFLUENZA

PREDICTIVE SPATIAL MODELLING OF HIGHLY PATHOGENIC AVIAN INFLUENZA SUBTYPE H5N8 IN FRANCE, 2016-2017

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SUMMARY

Beginning in 2014, Highly Pathogenic Avian Influenza (HPAI) H5N8 virus spread across Europe, causing unprecedented epidemics. In winter 2016, France was massively affected, resulting in culling of over 6 million poultry. Poisson Boosted Regression Trees (BRT) models were used to test for evidence of risk factors of HPAI-H5N8 infection in poultry holdings, and to predict and map the proportion of infected poultry holdings. Three datasets were combined to train the model: a dataset of the reported locations of HPAI-H5N8 outbreaks in domestic poultry during the 2016-2017 French epidemic, a dataset of the locations of poultry holdings where HPAI-H5N8 virus has not been reported and a set of relevant agro-ecological factors, including people, poultry production and trade, and water bird habitat. Results demonstrate the key role of the '*foie gras'* production in HPAI-H5N8 virus persistence and showed that reviewing transport of fattening ducks and limiting contact with wild birds are promising options.

INTRODUCTION

In Europe, France experienced its first unprecedented epidemics of HPAI-H5N8, with the first official report of poultry on November 28, 2016. As of March 23, 2017, a total of 484 confirmed outbreaks in domestic poultry, with 52 cases of wild birds were reported. Beginning in early 2017, in response to the emerging HPAI epidemic, French authorities implemented control measures, based on the use of pre-emptive culling and stringent movement restrictions. Approximatively 6 million poultry were culled, with culling strategies of all poultry implemented within a 1-km radius circle centred on reported outbreaks, and of all outdoor duck flocks within a 3-10 km radius circle. The movements of poultry were also restricted from outside and within the surveillance and protection zones (except for poultry scheduled for immediate slaughter or ducks entering the force-feeding stage).

Previous studies in South East Asia have shown that the risk of HPAI infection in poultry was associated with anthropogenic factors, such as human population density and distance to roads, domestic waterfowl density and indicators of water presence (Gilbert & Pfeiffer, 2012). In France, conditions of HPAI-H5N8 spread and persistence may somewhat differ from those reported in South East Asia due to different types of poultry production systems. During the

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2016-2017 epidemic, most of the outbreaks were reported in fattening duck holdings located in southwest France, a region renowned for its '*foie gras'* and fattening duck production, accounting for more than 70% of the world production. The short production cycle and the small size of force-feeding units result in frequent movements of fattening ducks taking place between different premises, making contacts through vehicles and staff the most likely mean for infection. The hypothesis that the disease might also be transmitted through wild birds is supported by the outdoor production (EFSA, 2017), allowing contacts between fattening ducks and potentially infected wild birds.

So far, however, apart from the movements of ducks and the presence of wild birds close to infected poultry premises, the sources of infection remain elusive. It is unclear what underlying risk factors are involved in the spread of HPAI, making control strategies difficult to refine. The objectives of this study were, therefore, to identify the agro-environmental factors associated with increased risk of HPAI-H5N8 infection and to generate predictive suitability areas for HPAI-H5N8.

MATERIALS AND METHODS

Data collection

H5N8 poultry outbreaks: Data regarding all confirmed HPAI-H5N8 outbreaks in commercial domestic poultry holdings in France were obtained from the French Ministry of Agriculture. A confirmed outbreak was defined as the detection of at least one laboratory-confirmed HPAI-H5N8 infected animal (by virus isolation or PCR) in a poultry flock. Outbreak data were grouped at commune (smallest administrative unit in France, with a median area of 10 km^2) level over the period of November 2016 to March 2017, and geo-referenced using the commune centroid coordinates in which it occurred. These coordinates were obtained from GEOFLA® (http://professionnels.ign.fr/geofla). While communes with one or more HPAI-H5N8 outbreaks were considered as positive data, pseudo-negative data were generated based on two conditions: having a number of poultry holdings > 1 to exclude communes without poultry production, and no HPAI-H5N8 outbreaks had been reported.

Spatial predictor variables: Two sets of variables were considered to influence the spatial distribution of HPAI-H5N8 outbreaks (Table 1). The first set (Set 1) included three variables related to hosts. The human population density per commune was generated from GEOFLA® (http://professionnels.ign.fr/geofla). Moreover, the density of chicken and duck holdings per commune were computed from the SIGAL and CIFOG databases. The second set (Set 2) included nine variables related to duck production and water bird habitat (Table 1). The density of holdings with ducks raised for meat, of fattening duck holdings and of duck holdings with outdoor access per commune were computed from the SIGAL and CIFOG databases. The density of incoming and outgoing fattening duck movements (from the breeding stage and directed to the force-feeding stage) per commune was computed from the CIFOG database over the period of October 2016 to February 2017. The density of force-feeding units and poultry slaughter houses per commune was computed from the DGAl database. Data on the distribution of wild bird species is generally coarse, with population demographics varying strongly according to the season. Therefore, indicators of water presence were used as proxy variables to discuss the possible effects of wild birds in HPAI-H5N8 outbreaks distribution. The density of waterways (i.e., rivers, streams, canals, etc. with length > 20 m) and the distance between the commune centroids and the closest water bodies (i.e., lakes, swamps, reservoirs, floods, etc. with area > 25 ha) were thus generated from BD CARTO®

(http://professionnels.ign.fr/bdcarto). ArcGIS 10.4 software (ESRI) and R software version 3.4.2 (R Development Core Team, 2011) were used for manipulation of spatial data.

Table 1. Predictor variables used in the BRT models.

Set	Variables	Abbreviation	Source
1	Density of human population	Dn_Pop	GEOFLA (IGN)
	Density of chicken holdings	Dn_Chick	SIGAL (DGAl)
	Density of duck holdings	Dn_Duck	CIFOG, SIGAL (DGAl)
2	Density of holdings with ducks raised for meat	Dn_Duck_Meat	SIGAL (DGAl)
	Density of fattening duck holdings	Dn_Duck_Fat	CIFOG, SIGAL (DGAl)
	Density of fattening duck holdings with outdoor access	Dn_Duck_Fat_Out	CIFOG
	Density of incoming fattening duck movements	Dn_InMvt	CIFOG
	Density of outgoing fattening duck movements	Dn_OutMvt	CIFOG
	Density of force-feeding units	Dn_FFunit	CIFOG
	Density of poultry slaughter houses	Dn_Sl	DGAl
	Density of waterways	Dn_Hydro	BD CARTO (IGN)
	Distance between the commune centroids and the closest water bodies	Dist_Hydro	BD CARTO (IGN)

Data analysis

Boosted regression tree models: Poisson Boosted regression tree (BRT) models (Elith et al., 2006, De'ath, 2007; Elith et al., 2008) were used to test for evidence of risk factors of HPAI A (H5N8) infection in poultry and to predict and map the proportion of infected poultry holdings. The number of infected poultry holdings was used as a dependent variable with an offset term corresponding to the total number of poultry holdings per commune.

BRT models were developed using the 4-fold cross-validation (CV) (Elith et al., 2008) to determine the optimal number of trees, limit overfitting and test the extrapolation capacity of the models. However, because the selection of data for the training and test sets is made at random, the resulting sets are not independent because of the spatial clustering of HPAI outbreaks and the presence of spatial autocorrelation through the predictor variables. This can lead to overestimating the goodness-of-fit (GOF) of the models which biases the evaluation of the model capacity to make predictions to independent datasets. Therefore, spatial CV was implemented, whereby the dataset was partitioned into four spatial blocks of insofar as possible equal numbers presence points, to measure the spatial extrapolation capacity of the models (Muscarella et al., 2014; Dhingra et al., 2016). Finally, the procedure was repeated 30 times to account for sources of uncertainty in data splitting. The GOF of the BRT models was assessed by calculating the Pearson correlation coefficient between the predicted and the observed response.

The relative contribution (RC) of each predictor variable, estimated from the identified number of trees, was used as a measurement of the importance of each variable to predict the proportion of infected holdings. BRT profiles were generated to determine the relationship between the predictor variable and the proportion of infected holdings. The mean predicted proportion of infected poultry holdings per commune was generated and mapped over the entire France. Models were run in R software version 3.4.2 (R Development Core Team, 2011) using dismo package (Elith et al., 2008).

Multi-collinearity: Variables related to poultry production showed multi-collinearity that may influence the contribution and profile of individual variables in the BRT models. Therefore, a stepwise approach for the selection of predictor variables was used to build the BRT models. A null model was first fitted to the four variables of the Set 1 (Table 1), to which were individually added the variables of the Set 2 tested in independent models. Then, the GOF metrics of the models were compared to those of the null model using a one-way ANOVA test. Ultimately, the final model was built with the four predictor variables of the null model and the predictor variables of the models found to significantly increase the accuracy of the model null.

Spatial scale: Southwest and northwest France are two main regions for fattening duck production, but most of the outbreaks were reported in fattening duck holdings located in southwest France. Consequently, two spatial scales were used to identify risk factors that explain the distribution of outbreaks in southwest France. First, the communes being at a maximum distance < 25 km of any positive (local model), or being at a maximum distance < 150 km of any positive (regional model) were selected to run the BRT.

RESULTS

The GOF metrics of the regional and local models for the different predictor variables and cross-validation methods are shown in Fig. 1. For the regional model, the combination of Set 1 with the density of outgoing movements (Mean Standard CV coefficient (StCV)= 0.67, Mean Spatial CV coefficient (SpCV)= 0.57) or with the density of fattening duck holdings with outdoor access (StCV = 0.60, SpCV = 0.52) or with the density of fattening duck holdings (StCV = 0.60, SpCV = 0.52) or with the density of incoming movements (StCV = 0.62, SpCV = 0.50) or with the density of waterways (StCV = 0.63, SpCV = 0.55) did result in significantly better models than Set 1 alone (StCV = 0.57, SpCV = 0.47) using both standard and spatial CV (P-value < 0.005). As a result, these five variables were kept in the final regional BRT model. For the local model, the combination of Set 1 and the density of outgoing movements (StCV = 0.70, SpCV = 0.65) did result in a significantly better model than Set 1 alone (StCV = 0.66, SpCV = 0.62) using both standard and spatial CV (P-value < 0.005). As a result, this variable was kept in the final local BRT model.

The relative contribution (RC) of the predictor variables for the final regional and local BRT models are shown in Table 2. In the regional model, the density of outgoing movements of fattening ducks showed the highest RC (50.6%), followed by the density of fattening duck holdings (20.5%). Lower RC were observed for the density of waterways (6.5%), the density of incoming fattening duck movements (6.3%), the density of duck holdings (5.3%) and the density of fattening duck holdings with outdoor access (4.9%). In the local model, the density of outgoing movements of fattening ducks also showed the highest RC (57.4%), followed by the density of duck holdings (28.6%). Lower RC were observed for the density of human

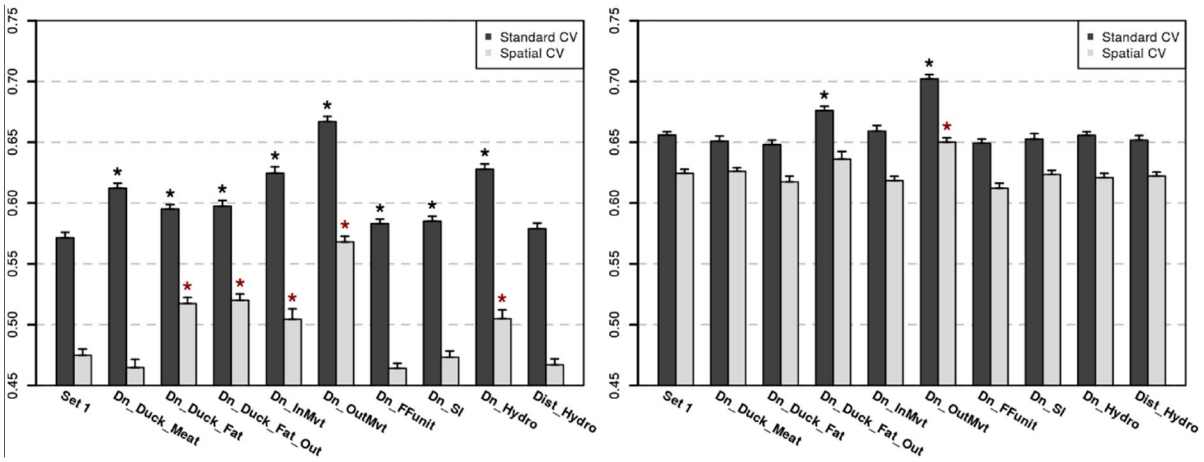

Fig. 1 Representation of Pearson coefficient correlation values (average and standard errors) for regional (left panel) and local (right panel) BRT models for the predictor variables using standard cross validation (CV) (in dark grey) and spatial CV (in light grey). The stars represent a significant difference in Pearson coefficient correlation values between the Set 1 and a variable using standard cross validation (CV) (in black) and spatial CV (in red).

Table 2. Average relative contribution (%) and standard deviation of predictor variables for the final regional and local BRT models.

Set	Predictor variable	Relative contribution (RC) (%)			
		Mean	Standard deviation	Mean	Standard deviation
		Regional model		Local model	
1	Density of human population	2.8	0.3	7.7	0.6
	Density of chicken holdings	3.1	0.5	6.3	0.5
	Density of duck holdings	5.3	0.9	28.6	0.7
2	Density of fattening duck holdings	20.5	0.7	-	-
	Density of fattening duck holdings with outdoor access	4.9	0.5	-	-
	Density of incoming fattening duck movements	6.3	0.5	-	-
	Density of outgoing fattening duck movements	50.6	2.6	57.4	1.8
	Density of waterways	6.5	0.6	-	-

population (7.7%) and the density of chicken holdings (6.3%). It is worth noting that the RC of the density of fattening duck holdings with outdoor access, of the density of incoming fattening duck movements and of the density of waterways decreased in the final BRT model, when considered in conjunction with the density of outgoing fattening duck movements and the density of fattening duck holdings.

The BRT profiles of the top-four predictor variables for the final regional and local BRT models are shown in Fig. 2. In both models, the density of outgoing movements of fattening ducks was positively associated with the proportion of infected holdings, with the maximum proportion approximately reached from 0.015 movements per ha. In the regional model, the proportion of infected holdings also increased strongly with the density of fattening duck holdings up to a value of 0.005 and slightly with the density of waterways up to a value of 1.2. In the local model, the density of duck holdings was positively associated with the proportion of infected holdings, with the maximum proportion approximately reached from 0.005 per ha. The density of human population and the density of chicken holdings were negatively associated with the proportion of infected holdings.

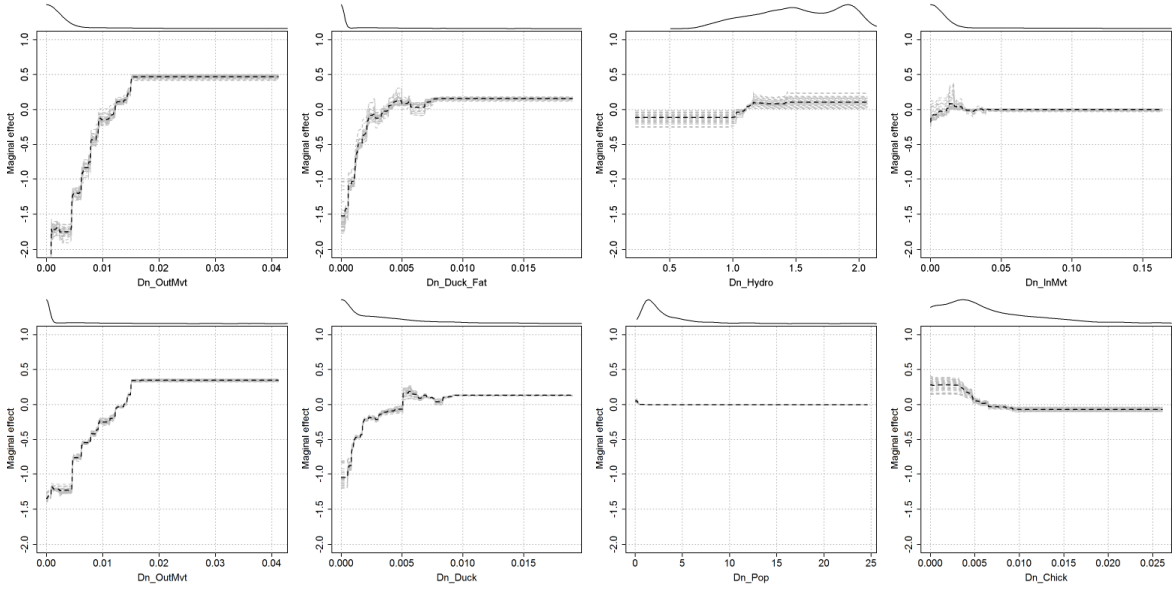

Fig. 2 BRT profiles of top-four predictor variables for the final regional (top panel) and local (bottom panel) BRT models. The grey lines represent the estimate for each bootstrap and the black line is the mean over all bootstraps. The lines at the top of plots show the distribution of data of the variable on the X-axis.

The HPAI-H5N8 suitability maps for the final regional and local BRT models are shown in Fig. 3. As expected, high proportion of infected holdings were predicted in southwest France for each model to varying extents. The regional model predicted that the proportion of infected holdings was low in northwest France where HPAI-H5N8 was introduced but did not persist during the 2016-2017 epidemics.

The distribution maps of the predictor variables are shown in Fig. 4. In southwest France (local scale), where most outbreaks occurred, poultry production is largely dominated by fattening duck holdings, particularly those with outdoor access. This area is also characterised by a high number of outgoing movements and a high density of waterways. In comparison,

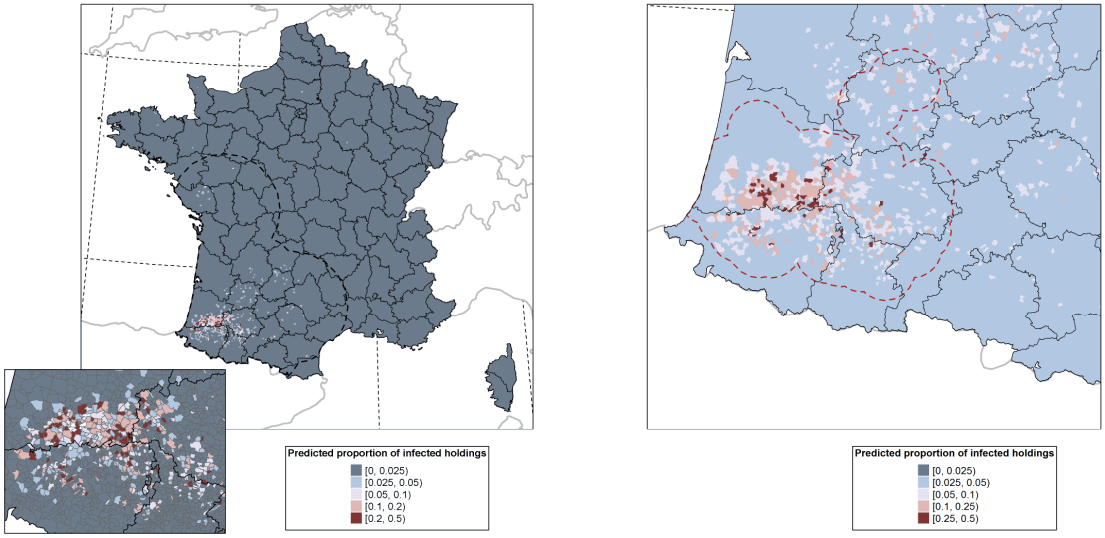

Fig. 3 Predicted proportion of HPAI-H5N8 infected poultry holdings for the final regional and local BRT models. The dashed black and red lines represent the regional and local scales, respectively.

northwest France, where limited number of outbreaks occurred, poultry production is largely dominated by holdings with chicken and with ducks raised for meat. These predictors in addition to human population represented lower predictors since they were more representative of northwest France.

DISCUSSION

As with a previous study (Hill et al., 2015), the results indicate that the duck holding density was positively associated with the proportion of infected holdings by HPAI-H5N8. The chicken holding density was negatively associated with the proportion of infected holdings, which may be mainly explained the reduced number of flock movements in the chicken production compared to those in the fattening duck production. Additionally, this could be explained by the hypothesis that chickens could be less susceptible to HPAI-H5N8 virus infection than ducks (Bertran et al., 2016). While human population density was often reported as one of the top predictors for other HPAI-H5 viruses' infection (Gilbert & Pfeiffer, 2012), it was here negatively associated with the proportion of infected holdings but with a low RC. This variable is often used as a proxy of higher likelihood of outbreak detection and of higher likelihood of transmission through poultry-related trade. Here it may rather reflect the area of southwest France where HPAI-H58 emerged, with relatively low human demographics (Fig. 4).

The results from the regional model further emphasised that among duck production, the fattening duck production was more related to HPAI-H5N8 outbreaks occurrence than the meat duck production. Indeed, four variables, that reflect the traditional fattening duck and *'foie gras'* production in southwest France, were positively associated with the proportion of HPAI-H5N8 outbreaks: the density of outgoing and incoming movements of fattening ducks from the breeding stage to the force-feeding stage, the density of fattening duck holdings with

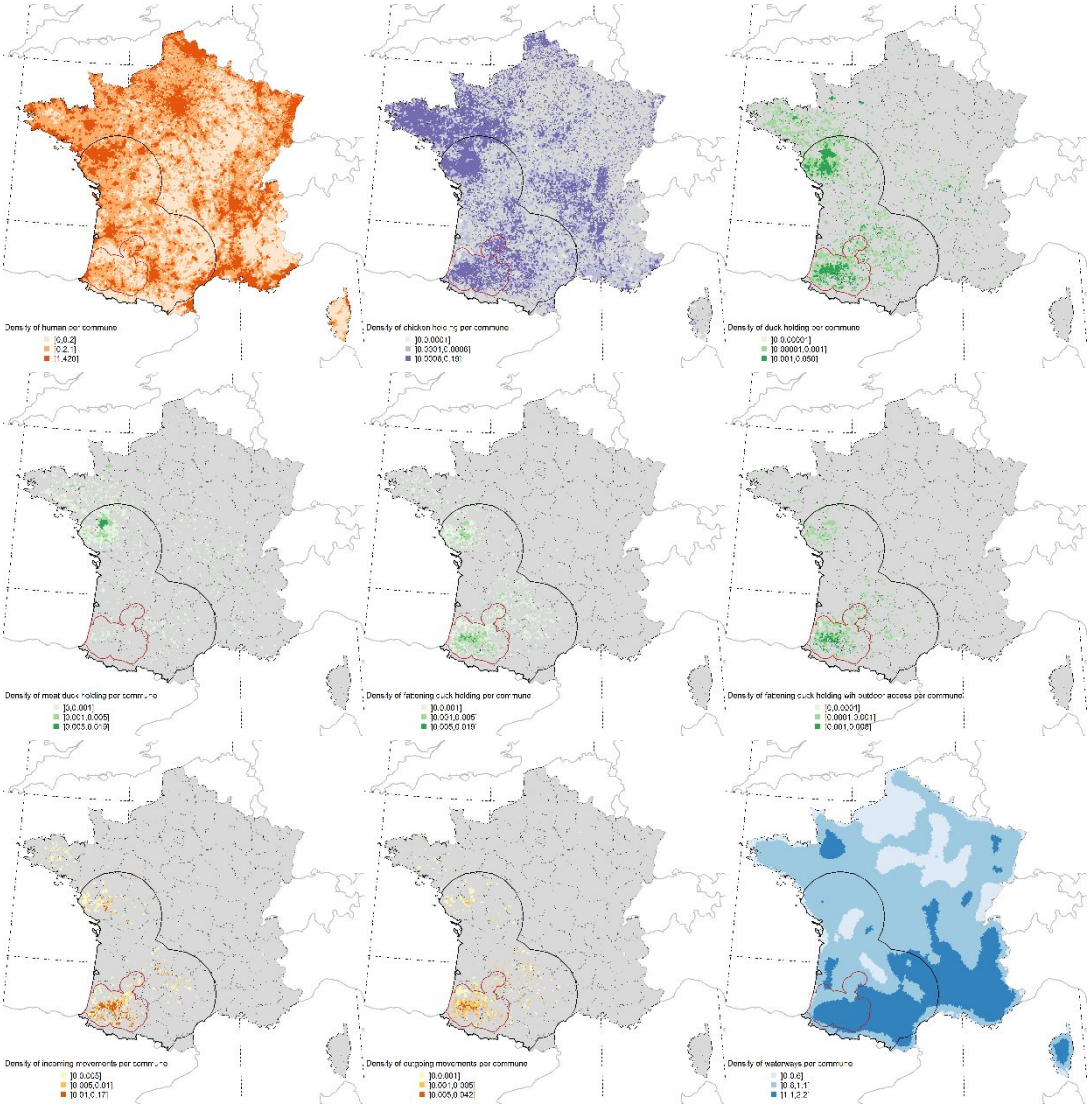

Fig. 4 Geographical distribution of predictors variables used in the final regional and local BRT models. The black and red lines represent the regional and local scales, respectively.

outdoor access and the density of waterways. Fattening ducks are bred in flocks of several thousand for up to 12 weeks, which are then frequently divided into small batches of hundreds of ducks to be moved to force-feeding units for 12 days, facilitating direct and indirect contacts between fattening ducks along transport networks (EFSA, 2017). Flocks of fattening ducks are also frequently raised on open land surfaces, facilitating direct and indirect contacts with wild birds that come to feed on duck's feeding trays (EFSA, 2017). The density of waterways from rivers and streams was used as a proxy of favourable habitats for various wild birds. This may support the hypothesis that holdings that share habitat with wild birds may be more likely to be infected with HPAI-H5N8, as they do for other HPAI-H5 viruses (Martin et al., 2011; Pandit et al., 2013). These results are interesting since they reflect major regional differences in fattening duck production between northwest and southwest France, combined with wild bird population conditions, with a consequential impact on the spatial distribution of HPAI-H5N8 outbreaks. Assuming similarities in the fattening duck production, this could also explain why outbreaks were also mostly identified in fattening duck holdings in Bulgaria and Hungary

(Bányai et al., 2016; Marinova-Petkova et al., 2016; EFSA, 2017) that are the second and third largest '*foie gras*' producers in Europe.

However, the results from the local model showed that only the density of outgoing movements of fattening ducks remained strongly associated with the proportion of infected holdings. This demonstrates that this variable was not only representative of regional differences related to fattening duck production, but rather would fully explained the outbreaks occurrence in southwest France. This stresses the need to further explore the role of movements of fattening duck in the HPAI-H5N8 outbreaks occurrence. The low contribution of the density of fattening duck holdings with outdoor access and the absence of the density of waterways, could be explained by their homogeneous geographical distribution in southwest France (Fig. 4).

Recently developed for predicting the distributions of organisms in ecological studies (Leathwick et al., 2006; Sinka et al., 2010), BRT approaches are very convenient in the interpretation of the results. The model outputs provide a profile of the effect of each predictor variable on the response variable over the range of its values (Fig. 2). This allows to determine the ranges of values of the predictor variables over which the effect was most important. Such information supports the design of control strategies, for example, based on the geographical location of fattening duck holdings, in particular those that are specialised in force-feeding ducks. In addition, the stepwise approach used here for the selection of predictor variables allowed us to increase the number of variables that were significantly related to the proportion of HPAI-H5N8 outbreaks and finally, to decrease the type II errors. Variables, such as the density of fattening duck holdings with outdoor access, the density of incoming fattening duck movements and the density of waterways that were masked because of collinearity among variables, were identified for the final BRT models.

In conclusion, the results highlight that the presence of fattening duck farming and water bird habitats may have strongly contributed to HPAI-H5N8 outbreak occurrence in southwest France. This suggests that preventing future poultry infections should rely on reviewing the movements of fattening ducks and limiting the contacts of fattening ducks with wild birds on open land. The suitability maps on the regional scale give an overall picture of HPAI-H5N8 risk in France that could be used to inform a national disease-control strategy and to allocate resources to French administrative departments with higher risk. Those on the local scale allow to refine the HPAI-H5N8 risk maps, with the aim to organise intervention at the commune level by veterinary officers. These findings are of particular importance to other fattening duck-producing countries affected by HPAI.

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NOMADIC MOVEMENTS AND INFECTIOUS DISEASE TRANSMISSION: TACKLING HIGHLY PATHOGENIC AVIAN INFLUENZA VIRUSES ALONG THE FREE-GRAZING DUCK MOVEMENT NETWORK IN VIETNAM

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SUMMARY

The presence of free-grazing ducks (FGD) has consistently been shown to be associated with highly pathogenic avian influenza (HPAI) outbreaks in South-East Asia. To provide a quantitative evaluation of their role and generate control recommendations, various stakeholders involved in the Vietnamese FGD production system were interviewed and a risk assessment model of HPAI transmission developed. These activities highlighted that duck flocks are transported extensively across district and province boundaries with almost one third of the grazing sites being located outside the province of residence. The mean probability of HPAI transmission between an infected flock to at least one susceptible flock during a grazing cycle was estimated at 0.28 (95% CI = 0.17 – 0.52). Transmission via indirect contacts during transport was identified as the most likely transmission route. Increasing vaccination coverage or increasing frequency of transport vehicle disinfection were identified as the two most effective strategies for decreasing the transmission risk.

INTRODUCTION

For over a decade, regular outbreaks of highly pathogenic avian influenza (HPAI) have occurred in poultry throughout South-East Asia, in spite of large-scale vaccination campaigns, as implemented in Vietnam and Indonesia, and stamping-out interventions (Brown, 2010). A large number of studies have contributed to greatly improve understanding of the epidemiology of HPAI viruses by highlighting the importance of several drivers of their distribution and spread. Noticeably, the presence of ducks was regularly shown to be strongly associated with the distribution of H5N1 outbreaks in Vietnam and in the wider region (Gilbert & Pfeiffer, 2012). In addition, live bird markets have been shown to contribute to the spread of avian influenza viruses and facilitate their persistence (Fournié et al., 2012). While formal live bird markets have been prohibited in the Mekong region, it is likely that there is continuing informal live bird trading activity, but it is recognised that this alone cannot explain the continuing circulation of avian influenza in South Vietnam.

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In Vietnam, poultry production in general, and duck production in particular, is concentrated in the Red River delta and the Mekong River delta (MRD). Free-grazing duck (FGD) farming is a common practice in these two regions, and about half of the duck production of the country originates from free-grazing management systems (Henning et al., 2013). In such systems, adult ducks scavenge freely on recently-harvested rice paddies where they feed on leftover rice grains, insects and molluscs. Two types of FGD systems have been described in Vietnam: *short-distance* (also called *stationary*) FGDs are herded within the commune boundaries and return to the farm premises at night, while *long-distance* (also called *moving*, *nomadic*, or *transhumant*) FGDs are herded outside the farm for an extended period of time, often several weeks, and are transported across administrative boundaries (Henning et al., 2013).

It has recently been suggested that long-distance FGDs are likely to play an important role in the maintenance and spread of avian influenza (AI) viruses (Henning et al., 2016). First, free-grazing ducks may act as local reservoirs and amplification hosts of virus transmitted by migratory birds, followed by secondary spread to other domestic poultry. Second, when released in the field for grazing or when being transported from one grazing place to another, long-distance FGDs may be in direct or indirect contact with other free-grazing duck flocks, potentially leading to virus transmission events. Finally, transport of FGD flocks across province and even national borders may lead to the spread of influenza viruses over relatively long distances.

By collecting field information amongst stakeholders involved in the Vietnamese FGD production system and developing a risk assessment model of HPAI virus transmission, this study aimed at i) describing the farming practices and contact patterns of long-distance FGD flocks in South Vietnam, ii) assessing the relative importance of different HPAI virus transmission routes between FGD flocks and iii) estimating the effectiveness of different control strategies to decrease the transmission risk.

MATERIALS AND METHODS

The study was conducted in An Giang province, in South Vietnam. Two districts (Chau Phu and Tri Ton) were selected on the basis of the importance of the integrated rice-duck production system and the presence of FGD flocks at the time of the field work, which took place between October and December 2015.

Field data collection

There are no official records of the location and movements of FGD flocks, so the only way to identify farmers to be interviewed was to use the most up-to-date local knowledge of communal veterinarians (Henning et al., 2013). Using this convenience sampling approach, 44 long-distance FGD farmers were identified and interviewed. In addition, 23 rice paddy owners and 17 FGD transporters were interviewed, and were identified based on the local knowledge of the commune veterinarians and of the FGD farmers already interviewed.

Three questionnaires, one for each type of stakeholder, were developed and tested in the field during a two-day pilot study. The farmer questionnaire included questions related to the socio-economics of the farm, duck production, flock movements and poultry health. For the purpose of this study, a flock was defined as a group of birds of the same age purchased, managed and sold as a whole. Farmers were asked to locate all sites that they had visited with their current flock. Subsequently, further aspects about each site (observed contacts between

flocks, type of transport used to travel between sites) were investigated. The location of each site specified by the farmers was approximated by the coordinates of the centroid of the commune where the site was located. The distance between sites was calculated as the Euclidean distance between two sites visited consecutively by a farmer.

The paddy owner questionnaire included questions related to the socio-economics of the household, the characteristics of the paddy management in terms of rice culture and post-harvest renting as well as the observed contacts between FGD flocks. The transporter questionnaire included questions related to the socio-economics of the household, the characteristics of the duck transport activity and the biosecurity practices.

Risk assessment workshop

To assess the relative importance of different HPAI virus transmission routes between FGD flocks and estimate the effectiveness of different control strategies to decrease the transmission risk, a stochastic risk assessment model tailored to the context of HPAI in South Vietnam was developed. The risk assessment was framed around the following risk question: *"With current management practices of free-grazing duck flocks, what is the risk of one HPAI-H5N1-infected long-distance free-grazing duck flock transmitting the virus to at least one susceptible free-grazing duck flock during a grazing cycle?"* The risk assessment considered several pathways of exposure of a susceptible FGD flock to H5N1 virus from an infected FGD flock to account for the diversity of transmission routes that might occur in the Mekong Delta region.

As a preliminary step, all potential pathways were discussed at a risk assessment workshop held in Hanoi in April 2015. This workshop brought together around 30 people representing a wide range of Vietnamese stakeholders including members of the regional and provincial divisions of the Department of Animal Health (DAH) of the Ministry of Agriculture as well as local representatives of the Food and Agriculture Organization of the United Nations (FAO). Based on the workshop discussions, the risk assessment focused on the six most relevant exposure pathways. These pathways included: exposure by 1) direct and 2) indirect contact between duck flocks in the field when grazing, exposure by 3) direct and 4) indirect contact in boats when transported from a grazing site to another, exposure by 5) direct and 6) indirect contact in trucks when transported from a grazing site to another (Fig. 1).

The risk assessment workshop was also used to define with the participants the different intervention strategies that should be investigated as part of the risk assessment modelling study, in order to control the spread of H5N1 virus along the free-grazing duck movement network (see below).

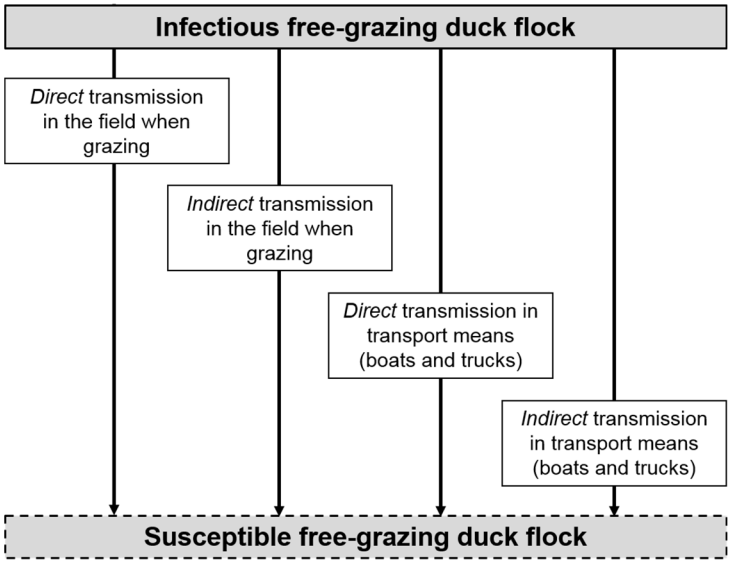

Fig. 1 Main risk pathways for the transmission of avian influenza viruses between long-distance free-grazing duck flocks in the Mekong region, Vietnam.

Risk assessment model

The overall probability *P* that an infectious long-distance FGD flock transmits H5N1 virus to at least one susceptible FGD flock during a grazing cycle was calculated as shown in Eq. (1).

$$P = 1 - \prod_{i=1}^{6}(1 - P_i) \quad (1)$$

with P_i being the probability that an infectious long-distance FGD flock transmits H5N1 virus to at least one susceptible FGD flock during a grazing cycle through the transmission pathway *i*. The next paragraphs describe how the different P_i were calculated.

Transmission by direct contact in the field: This transmission pathway assumes that transmission could occur either because a few ducks from the infectious flock mingle temporarily with a susceptible flock or vice versa. Consequently, the probability that an infectious FGD flock transmits H5N1 virus to at least one susceptible FGD flock during a grazing cycle by direct contact in the field (P_1) was calculated as shown in Eq. (2).

$$P_1 = 1 - (1 - P_{1a}) * (1 - P_{1b}) \quad (2)$$

with P_{1a} being the probability that some birds from the infected flock are temporarily integrated into a susceptible flock and transmit the virus to at least one duck before being brought back to the infectious flock and P_{1b} being the probability that some birds from a susceptible flock are temporarily integrated into the infected flock and at least one becomes infected before being brought back to the susceptible flock. P_{1a} and P_{1b} were calculated as shown in Eq. (3) and (4), respectively.

$$P_{1a} = \alpha * \beta * \gamma * (1 - (1 - \pi)^n) * (1 - \nu) * \delta \quad (3)$$

$$P_{1b} = \alpha * \beta * (1 - \gamma) * (1 - \nu) * \varepsilon \quad (4)$$

with α being the probability that two FGD flocks graze simultaneously in the same or neighbouring fields, β being the probability that two flocks grazing in the same or neighbouring fields get in direct contact (i.e. a few birds from one flock are temporarily integrated into the other flock), γ being the probability that the visiting ducks are from the infectious flock, π being the within-herd prevalence of HPAI in the infected flock, n being the number of ducks that are temporarily integrated into the other flock (so $(1-(1-\pi)^n)$ being the probability that at least one infected duck is temporarily integrated into the susceptible flock), ν being the probability that the other FGD flock is vaccinated (so $(1-\nu)$ is the probability that the other flock is susceptible), δ being the probability that at least one duck from the susceptible flock becomes infected given n ducks from the infectious flock were temporarily integrated into the susceptible flock and ε being the probability that at least one susceptible duck temporarily integrated into the infectious flock becomes infected.

Transmission by indirect contact in the field: This transmission pathway assumes that transmission could occur because the same harvested rice field can be used successively by two FGD flocks before the new rice production cycle starts again and because HPAI virus has been shown to be able to survive for several days in the environment, water and faeces (Lebarbenchon et al., 2010; Kurmi et al., 2013). Consequently, a susceptible FGD flock could become infected if it visits the grazing site before the virus that had been excreted by the previous infectious flock is inactivated. Given that the duration of the between-harvest period is generally no longer than three weeks, it was assumed that only a maximum of one indirect contact between flocks could occur per grazing cycle. Consequently, the probability an infectious FGD flock transmits H5N1 virus to at least one susceptible FGD flock during a grazing cycle by indirect contact in the field (P_2) was calculated as shown in Eq. (5).

$$P_2 = \zeta * \eta * \theta * (1-\nu) * \kappa * \lambda \quad (5)$$

with ζ being the probability that some H5N1 virus is excreted in the environment by the infectious flock, η being the probability that two FGD flocks graze successively on the same rice paddy field between two rice production cycles, θ being the probability that the first visiting FGD flock is the infectious one, ν being the probability that the second visiting FGD flock is vaccinated, κ being the probability that the susceptible flock arrives before the excreted virus is inactivated (this period was assumed to be five days) and λ being the probability that the susceptible flock becomes infected given there is still infectious virus in the rice paddy field.

Transmission by direct contact in boats: This transmission pathway assumes that transmission could occur because different flocks can be transported together in the same boat from one grazing location to the next. Consequently, the probability that an infectious FGD flock transmits H5N1 virus to at least one susceptible FGD flock during a grazing cycle by sharing the same boat (P_3) was calculated as shown in Eq. (6).

$$P_3 = \mu_{boat} * \xi_{boat} * (1-\nu) * o_{boat} \quad (6)$$

with μ_{boat} being the probability that the infectious flock is transported by boat (as opposed to by truck or by foot), ξ_{boat} being the probability that two flocks are transported together in the same boat, ν being the probability that the other FGD flock is vaccinated, and o_{boat} being the probability that at least one duck from the susceptible FGD flock becomes infected during transport if it is transported together with an infectious flock in the same boat.

Transmission by indirect contact in boats: This transmission pathway assumes that transmission could occur because the same boat can be used to transport successively several FGD flocks without being cleaned nor disinfected and because HPAI virus has been shown to be able to survive for several days in the environment. Since a boat could complete several journeys during the survival period of the virus, the frequency of boat journeys needed to be accounted for. Consequently, the probability an infectious FGD flock transmits H5N1 virus to at least one susceptible FGD flock during a grazing cycle by direct contact in a boat was calculated as shown in Eq. (7).

$$P_4 = \mu_{boat} * (1 - (1 - (1 - \nu) * \tau_{boat})^{m_{boat}}) \quad (7)$$

with μ_{boat} being the probability that the infectious flock is transported by boat, ν being the probability that the second FGD flock is vaccinated, τ_{boat} being the probability that at least one duck from the susceptible FGD flock becomes infected during transport if the boat is contaminated and m_{boat} being the number of boat journeys completed before the boat is cleaned and disinfected or the virus is deactivated.

Transmission by direct and indirect contacts in trucks: To calculate the probabilities that an infectious FGD flock transmits H5N1 virus to at least one susceptible FGD flock during a grazing cycle by direct or indirect contact in a truck, similar formulations than those of the probabilities for direct and indirect contacts in boats were used with truck-specific probabilities when necessary.

Model calibration, simulations and sensitivity analysis

Most of the model was parameterised using the data generated by the interviews of FGD farmers, rice paddy owners and FGD transporters. Other parameter values not tailored to the context of South Vietnam were adapted from the literature. Table 1 presents all the model parameters together with the values or distributions used in the simulations and the associated references. This defined the baseline scenario.

The distribution of the overall probability *P* that an infectious long-distance FGD flock transmits H5N1 virus to at least one susceptible FGD flock during a grazing cycle was determined by sampling 1,000 parameter values in their respective distributions and combining them as described in the previous paragraphs.

All analyses were performed using the software @Risk7 (Palissade Corporation, USA).

Effectiveness of control strategies

To assess the relative impact of different control strategies on the overall probability *P*, some input parameters were modified to simulate different interventions potentially applicable in the field. The set of control strategies to be assessed were defined by local and national stakeholders who participated in the risk assessment workshop. They included 1) an increase of the vaccination coverage, 2) and 3) an increase of the frequency of cleaning and disinfection of boats and trucks, respectively, and 4) and 5) a reduction of the frequency of mixing of flocks during transportation in boats and trucks, respectively. The impact of changing each of the corresponding input parameters, as summarised in Table 2, was evaluated separately in parallel simulations where all other parameters were assigned the values and distributions defined in the baseline scenario.

Table 1. Parameters used in the baseline scenario of HPAI transmission between long-distance free-grazing duck flocks (Par: parameter; RPO: rice-paddy owner; F: long-distance free-grazing duck farmer; T: long-distance free-grazing duck transporter; parameters with an asterisk (*) were given fixed values to simplify the comparison with the alternative scenarios).

Par	Description	Value	Reference
α	Probability that two FGD flocks graze simultaneously in the same or neighbouring fields	Beta(15,6)	Interviews (RPO)
β	Probability that two flocks grazing in the same or neighbouring fields get in direct contact	Beta(32,14)	Interviews (F)
γ	Probability that the visiting ducks are from the infectious flock	0.5	NA
π	Within-herd prevalence of HPAI	Beta(29,82)	Henning et al., (2010)
n	Number of ducks that are temporarily integrated into the other flock	Pert(1,10,100)	Interviews (F)
δ	Probability that at least one duck from the susceptible flock becomes infected given n ducks from the infectious flock were temporarily integrated into the susceptible flock	Pert(0.3,0.7,0.9)	adapted from Hernández-Jover et al., (2015)
ε	Probability that at least one susceptible duck temporarily integrated into the infectious flock becomes infected	Pert(0.4,0.8,0.9)	adapted from Hernández-Jover et al., (2015)
ν*	Probability that a FGD flock is vaccinated	0.7	DAH, pers. com.
ζ	Probability that some HPAI virus is excreted in the environment by the infectious flock	1	NA
η	Probability that two FGD flocks graze successively on the same rice paddy field between two rice production cycles	Beta(13,9)	Interviews (RPO)
θ	Probability that the first visiting FGD flock is the infectious one	0.5	NA
κ	Probability that the susceptible flock arrives before the excreted virus is inactivated (less than 5 days after the departure of the previous flock)	Beta(9,3)	Interviews (RPO)
λ	Probability that the susceptible flock becomes infected given there is still infectious virus in the rice paddy field	U(0.05,0.3)	adapted from Hernández-Jover et al., (2015)

μ_{boat}	Probability that the infectious flock is transported by boat	Beta(38,8)	Interviews (F)
ξ_{boat}*	Probability that two flocks are transported together in the same boat	0.3	Interview (T)
o_{boat}	Probability at least one duck from the susceptible FGD flock becomes infected during transport if it is transported together with an infectious flock in the same boat	Pert(0.3,0.7,0.9)	adapted from Hernández-Jover et al., (2015)
ρ_{boat}*	Average number of days between two cleaning and disinfection events in boats	7	Interview (T)
tcd_{boat}	Time to the next cleaning and disinfection event in boats (days)	U(0,Pois(ρ_{boat}))	NA
inf_{boat}	Time to the moment the boat stops being infectious (days)	min(5,tcd_{boat})	NA
σ_{boat}	Average daily frequency of boat journeys	1	Interview (T)
m_{boat}	Number of boat journeys implemented before the boat is cleaned and disinfected	Pois(inf_{boat} *σ_{boat})	NA
τ_{boat}	Probability at least one duck from the susceptible FGD flock becomes infected during transport if the boat is contaminated	U(0.05,0.3)	adapted from Hernández-Jover et al., (2015)
μ_{truck}	Probability that the infectious flock is transported by truck	Beta(10,36)	Interviews (F)
ξ_{truck}*	Probability that two flocks are transported together in the same truck	0.3	Interview (T)
o_{truck}	Probability at least one duck from the susceptible FGD flock becomes infected during transport if it is transported together with an infectious flock in the same truck	Pert(0.3,0.7,0.9)	adapted from Hernández-Jover et al., (2015)
ρ_{truck}*	Average number of days between two cleaning and disinfection events in trucks	14	Interview (T)
tcd_{truck}	Time to the next cleaning and disinfection event in trucks (days)	U(0,Pois(ρ_{truck}))	NA
inf_{truck}	Time to the moment the boat stops being infectious (days)	min(5,tcd_{boat})	NA
σ_{truck}	Average daily frequency of truck journeys	1	Interview (T)
m_{truck}	Number of truck journeys implemented during the survival period of the virus (assumed to be 5 days)	Pois(inf_{truck} *σ_{truck})	NA
τ_{truck}	Probability at least one duck from the susceptible FGD flock becomes infected during transport if the truck is contaminated	U(0.05,0.3)	adapted from Hernández-Jover et al., (2015)

Table 2. Description of the alternative control strategies ("ref" indicates the value that had been used in the baseline scenario).

ID	Description	Parameter modified	Values
1	Increase of the vaccination coverage	ν	0.7 (ref), 0.8, 0.9
2	Decrease the average number of days between two cleaning and disinfection events in boats	ρ_{boat}	7 (ref), 3.5, σ_{boat}
3	Decrease the average number of days between two cleaning and disinfection events in trucks	ρ_{truck}	14 (ref), 7, σ_{truck}
4	Reduction of the frequency of mixing of flocks during transportation in boats	ξ_{boat}	0.3 (ref), 0.2, 0.1, 0
5	Reduction of the frequency of mixing of flocks during transportation in trucks	ξ_{truck}	0.3 (ref), 0.2, 0.1, 0

RESULTS

Among 219 unique grazing sites reported by the 44 farmers during the interviews, 146 (67%) were located in the home province of the reporting farmer, while most of the remaining sites were in other Vietnamese provinces of the MRD apart from 2 (1%) which were located in Cambodia, close to the Vietnamese border. The median distance between two successive journeys was 30 km (standard deviation: 16 km, range: 1-85). The total distance travelled by each flock between the date when it was purchased and the date of the survey ranged between 21 and 763 km (median 125 km with standard deviation 140 km). Three means of transport were used by the farmers to move their flocks between paddies: out of the 209 journeys for which information was available, 137 (66%) were by boat, 25 (12%) by truck and 47 (22%) by foot. As the layer duck production cycle is relatively long (up to two years), FGD flocks return to their home commune up to six times during their life time.

As illustrated in Fig. 2, the probability P that an infectious long-distance FGD flock transmits H5N1 virus to at least one susceptible FGD flock during a grazing cycle was estimated at 0.28 (95% CI = 0.17 – 0.52). Transmission via indirect contacts during transport in boats or trucks $(1-(1-P_4)*(1-P_6))$ was identified as the most likely transmission route, contributing to 50% (95% CI = 6 – 82%) of the overall risk. Transmission via direct or indirect contacts when grazing in the rice paddy fields $(1-(1-P_1)*(1-P_2))$ contributed to 39% (95% CI = 19 – 65%) of the overall risk, while transmission via direct contact during transport through the mixing of flocks in boats or trucks $(1-(1-P_3)*(1-P_5))$ contributed to 22% (95% CI = 11 – 38%) of the overall risk.

From the analysis of the effectiveness of alternative control strategies came out that a vaccination increase from 70% to 90% of the FGD flocks could allow a reduction of the overall transmission risk by 63% (95% CI = 58 – 65%). Doubling the daily frequency of boat and truck cleaning and disinfection is expected to reduce the overall transmission risk by 12% and 4%, respectively. Halving the frequency of mixing of flocks during transportation in boats and trucks could decrease the transmission risk around 9%.

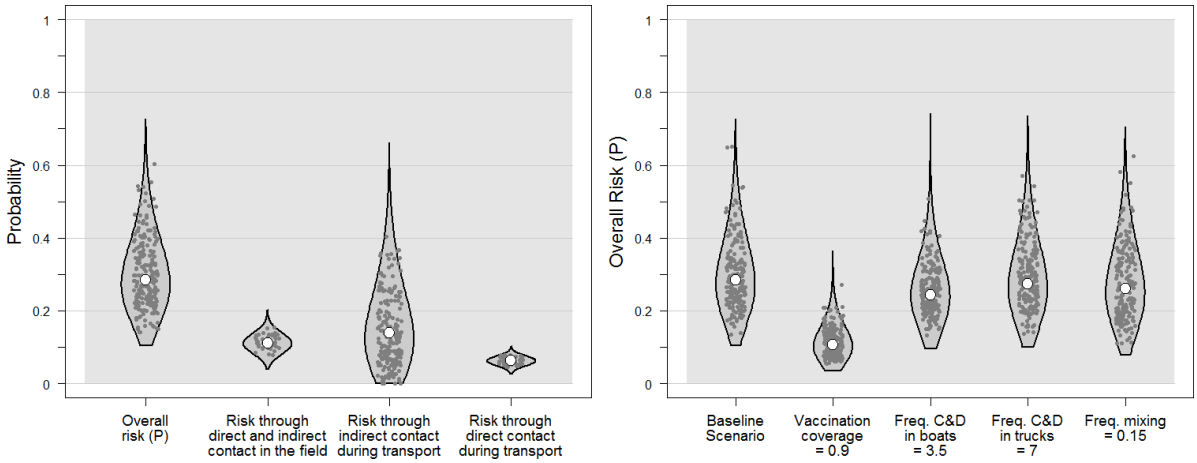

Fig. 2 Distribution of the risk of transmission of H5N1 virus between free-grazing duck flocks depending on the transmission route (left) and for different control strategies (right).

DISCUSSION

Although free-grazing duck production systems have been on the decline in South-East Asia for the last three decades as a result of intensification of agricultural production, increasing cost of labour and increasing pesticide use, it is still widely practiced in at least four countries: China, Indonesia, Thailand and Vietnam (Gilbert et al., 2006; Henning et al., 2013, 2016). This production system is characterised by intense and diverse contacts between duck flocks as well as long-distance journeys that could lead to the maintenance and spread of avian influenza viruses. The study presented here highlighted that indirect contacts between flocks during transportation was the most likely transmission route of H5N1 virus and quantified the impact several control strategies could have on the risk of disease transmission: an increase of the vaccination coverage and an increase of the frequency of boat and truck cleaning and disinfection being the two most effective strategies in reducing the risk.

Increasing the vaccination coverage of long-distance FGD flocks against H5N1 subtype was shown to be the most effective control strategy, as it likely affects all transmission routes by decreasing the probability the in-contact FGD flock is susceptible. Since 2012, vaccination is no longer compulsory in Vietnam. However, some provinces continue subsidising local vaccination campaigns by covering vaccine and labour costs. Vaccination generally involves commercial inactivated vaccines (Navet-Vifluvac®, Re-5 and Re-6) and represents the main preventive measure against HPAI H5N1 in ducks. It consists of two injections at a 3-week interval. This vaccination protocol makes vaccination campaigns very challenging for nomadic FGD flocks because they rarely stay more than four weeks at the same grazing location. As a consequence, most FGD flocks that engage in this vaccination strategy receive only one injection leading to incomplete protection. The true effective vaccination coverage of FGD flocks is difficult to estimate but is expected to be especially low in provinces that do not subsidise the vaccination campaigns. Consequently, vaccination protocols for nomadic FGD flocks should be improved by promoting inter-provincial collaborations of veterinary services in order to optimise the vaccination coverage of FGD flocks, but this is expected to be challenging.

A certification scheme promoting “clean transport vehicles” should be developed to reward transporters who would commit to clean and disinfect their transport vehicles on a regular basis.

Such an incentive system would be successful only if it is fully supported by both transporters and FGD farmers. This is expected to promote a shift amongst transporters towards good hygiene practices leading to a decrease of the risk of transmission of AIV through indirect contact during transport.

Sharing boats or trucks to transport several flocks together is mostly practiced by owners of small-size FGD flocks (<1000 ducks) to reduce the transport costs. This practice is expected to lead to direct contacts between different flocks and therefore promote the spread of AIVs between flocks. The simulation model highlighted that discouraging the transport of more than one flock per vehicle would not reduce substantially the risk of AIV transmission. Therefore, the strong economic rationale justifying this practice is expected to be very difficult to argue. Additionally, the current model does not explicitly account for flock size. Therefore, the estimated effectiveness of this strategy is likely to slightly overestimate its true effectiveness, as this strategy would mostly concern small-size flocks (<1000 ducks), which represent less than 40% of the duck flock population in South Vietnam (Henning et al., 2013; Meyer et al., 2017).

The results presented in this study served as the scientific basis for a policy workshop entitled "Understanding the role of free-grazing ducks in the circulation of influenza viruses A in Vietnam: from research to policy", held in Hanoi, Vietnam in March 2017. This workshop brought together around 40 people representing a wide range of experts and stakeholders including Vietnamese, European and Australian scientists, governmental authorities, members of the regional and provincial divisions of the DAH and representatives of the Food and Agriculture Organization of the United Nations (FAO). The objectives of the workshop were to examine and debate the different strategies that could be implemented to tackle avian influenza in FGD populations and their implications in terms of policy. In the context of a limited budget allocated to control of HPAI in Vietnam, it was agreed that priorities for additional activities should be given to the development of a certification scheme for “clean transporters” to prevent indirect transmission during transport in the Mekong river delta region.

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NETWORK ANALYSIS

COMBINING WHOLE GENOME SEQUENCING AND ANALYTIC CONTACT TRACING FOR IMPROVED DETECTION OF *SALMONELLA* DUBLIN TRANSMISSION ROUTES

L.V. DE KNEGT*, E. KUDIRKIENE, E. RATTENBORG, G. SØRENSEN, J.E. OLSEN AND L.R. NIELSEN

SUMMARY

Whole genome sequencing (WGS) of *Salmonella* Dublin was combined with information on cattle movements and with the grouping of cattle herds into business-units. The EpiContactTrace R-package was used to trace contacts between businesses sharing the same clones of *S.* Dublin. The aim was to investigate new explanations for *S.* Dublin circulation in groups of herds without recorded livestock movements. Results indicated that the use of businesses as epidemiological units for contact-tracing provided information that could not be obtained using herds as main units. However, this concept may overestimate real-life contacts between cattle properties, since it is only based on property ownership and location, putatively overestimating the degree of risk-contacts within each business. Still, the combination of WGS and the business approach was promising for future disease tracing tasks, and may provide evolutionary knowledge about *S.* Dublin, which can be applied for its improved control.

INTRODUCTION

Control programmes for infectious diseases in livestock rely heavily on restricting or controlling animal movements directly between farms (or regions), as well as transit through high risk areas, to reduce the potential for transmission by direct contact (Houe et al., 2014). This is a sensible strategy, as numerous studies have demonstrated the importance of trade networks and direct contacts between livestock as risk factors for transmission of disease (for example: Bigras-Poulin et al., 2006; Frössling et al., 2012; Mweu et al., 2013; Sintayehu et al., 2017).

An example of a control programme based heavily on restriction of movements is the Danish *Salmonella* Dublin programme, which aims to eradicate *S.* Dublin from the cattle population. The prevalence of test-positive dairy cattle properties has been reduced from 25% in 2002 to 7% in 2015, but it has proven difficult to reduce the apparent prevalence further, and new introductions of *S.* Dublin to cattle herds still occur despite strict regulation of cattle contacts

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and trade. Therefore, it is of interest to improve the understanding of the transmission mechanisms of *S*. Dublin.

In Denmark, movement of cattle between herds must be recorded in the Central Husbandry Registry (CHR), which is closely linked to the Danish Cattle Database (DCDB), maintained by SEGES (Anonymous, 2017). The systems differentiate between cattle herds (i.e. groups of animals belonging to the same owner) and properties in which there may be several herds and several species present. The owner of the property is often the same as the owner of the herds, but not always. Surveillance programmes usually test and assign health status at property level. Hence, all herds recorded at the same property will be assigned the same surveillance status.

In theory, it should be easy to trace infections that spread by movement of cattle. However, cattle farmers are excused from reporting cattle movements when their herds are registered as part of an official partnership (in Danish: 'samdrift') or a neighbour agreement (in Danish: 'naboaftale'). Partnerships can be registered when two or more herds belonging to the same owner are located at a maximum Euclidean distance of four kilometres from each other, and the properties where the herds are located do not contain ungulates belonging to another owner. Neighbour agreements can be made when animals of herds belonging to the same owner, but located in two neighbouring properties, are mixed in outdoor areas during the grazing season, moving freely between the two properties (shared pastures), and those properties contain herds of ungulates belonging to another owner. Herds cannot be part of a neighbour agreement and a partnership at the same time. Movements of cattle between herds belonging to the same owner, which are not part of a partnership, must be recorded normally (Anonymous, 2017). The system is constantly checked for errors as part of the European Union cross-compliance standards and demands, and penalties in the form of the reduction or exclusion of support received under the Common Agricultural Policy are applicable in cases of non-compliance (Anonymous, 2013). The data are, therefore, expected to be highly reliable.

The existence of concepts such as official partnerships and neighbour agreements reflects the cultural understanding that it is natural for neighbouring farms, properties or herds belonging to the same farmer to function as one farm, or one epidemiological unit. This understanding is supported by field observations, with properties owned by the same person and located at close proximity effectively functioning as one, sharing machinery, feed, personnel and animal circulation (Langvad et al., 2006; Nielsen et al., 2012). Such multisite structures and farm management habits are expected to have an impact on the farm biosecurity, and hence the potential transmission of infectious diseases, including *S*. Dublin.

The OIE (2015) currently defines biosecurity as "a set of management and physical measures designed to reduce the risk of introduction, establishment and spread of animal diseases, infections or infestations to, from and within an animal population". This definition acknowledges that management practices affecting animal contacts within farms also have potential to affect transmission of infectious diseases between different groups. However, within-farm biosecurity has hitherto mostly been perceived as a backup system, when between-farm biosecurity has failed. Therefore, the focus is still mostly on avoiding between-herd transmission or introduction of infections to naïve herds (Brennan & Christley, 2012).

Bigras-Poulin et al. (2006) observed that the animal movement network is, in fact, only one component of a larger network formed by different links through movements of animals, veterinarians, milk trucks, feed, machinery etc. During the investigation of an outbreak of *Salmonella* Typhimurium DT104 in Denmark in 1999 (Langvad et al., 2006), researchers observed animal feed being transported from infected to non-infected properties owned by the

same person. They also observed the sharing of machinery, slurry tanks and grazing areas between same- or differently-owned herds, which all became infected with the same clone of salmonella. The study concluded that, although animal movements between farms are generally the primary transmission route, transmission by people, equipment and other physical sources are also of great importance. It is, therefore, expected that these more diffuse transmission routes can occur between cattle properties belonging to the same owner, independent of whether they are in a registered official partnership or not. This would allow for circulation pathways of infectious agents, including *S*. Dublin bacteria, independent of animal movements. The authors' propose, therefore, that when tracing trade networks, larger epidemiological units should be used than the herd or property itself, so as not to miss the indirect links between herds in the tracing networks. Such multisite epidemiological units will, in this paper, be referred to as "businesses". The functional existence of such epidemiological units is obvious, based on anecdotal evidence and field observations, but data-based evidence of their importance in tracing of infectious diseases would be useful.

Whole genome sequencing (WGS)-based phylogenetic analysis enables the detection of closely-related clones of *S*. Dublin. WGS information has recently been used to investigate outbreaks of *S*. Dublin in Sweden (Ågren et al., 2016) and to detect local persistence and transmission patterns of genotypes of other pathogens in other countries (Biek et al., 2012, Trewby et al., 2016). A strain collection of *S*. Dublin isolates stored since 1996 at the Danish National Food Institute provided an opportunity to combine epidemiological tracing with WGS technology.

Hence, the objective here was to use WGS of stored *S*. Dublin isolates to substantiate pathogen circulation within and between businesses, to validate the proposal of considering businesses rather than individual herds (or properties) as epidemiological units, when analysing animal movement-based transmission patterns and tracing of infectious diseases.

MATERIALS AND METHODS

The general principle of the presented approach is analogue to an attempt to increase the sensitivity of contact-tracing analyses in outbreak investigations, by using businesses instead of individual properties or herds of cattle, to reduce the number of missed links between herds in the tracing. It is expected that a larger number of true contacts carrying a risk of transmission of infectious agents is detected for businesses than for properties or herds. However, even for businesses that do not make sense as an epidemiological unit, there would still be an increase in detected contacts, by the sheer logic of including more properties. Therefore, in order to support the epidemiological validity of the business concept, it is necessary to ensure that the increase in sensitivity would not result in a large number of non-risk-carrying contacts being suggested between herds. The steps taken to assess the effectiveness of tracing contacts using businesses versus properties were:

1) Trace cattle movements for properties from which *S*. Dublin isolates had been sequenced, and locate other properties with sequenced isolates, to observe the degree of relation between the clones found in the source and destination properties;
2) Trace cattle movements for businesses and locate other businesses containing properties with sequenced isolates;
3) Observe the number of detected contacts to assess the gain in contact detection, as well as the degree of genetic relation between those contacts, to assess losses in the quality of detection.

Details of each part of the process are described below.

Data sources and management

Cattle movement data: The movement records maintained by the CHR/DCDB refer to individual cattle movements between *herds*. A herd is formally defined as a group of animals of the same species and finality (slaughter, dairy, breeding, etc.), owned by the same person and located within the same property. A *property* may contain different herds, which may belong to different owners. Different herds within a property will normally be in physically separated facilities, and may contain different animal categories, species or finalities. Each cattle movement generates two records in the database: one outgoing from the source herd, and one ingoing to the receiving herd. In the dataset made available by SEGES, movements were described by the source and destination herds' property identifier (CHR number), herd number, owner ID (anonymised) and production type, plus date of movement and movement type (live animals, slaughter, export, import, birth, death, euthanasia, etc.). Moved animals were identified by their unique individual number, as well as other variables related to age, sex, breed and calving (if applicable). The dataset included 33 variables, and contained 71,593,850 records (corresponding to 35,796,925 cattle movements) from 1998 to 2017. Considering the objectives of this study, only live animal movements not for slaughter, import or export were selected from the movement dataset, and movements without proper identification of the herds involved were excluded. This resulted in 9,475,624 movements between 55,080 herds from 45,085 properties, characterized by variables describing the date of the movement, herd/owner/property of origin, and herd/owner/property of destination.

Genetic typing data: A total of 196 isolates of *S*. Dublin from cattle herds in 58 properties, collected on-farm in different parts of the peninsula of Denmark (Jutland) between 1996 and 2016 from surveillance and project activities, were stored at the National Food Institute, Technical University of Denmark. These were collected from the freezer, re-grown and sequenced (WGS) using 250 pair-end MiSeq (Illumina). Sequences were assembled with SPADes 3.9.1, and annotated using Prokka 1.0. The population structure was obtained using CSI Phylogeny, with recombinant regions excluded. Trees were also visually analysed using FigTree v1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/). This population was characterized by three main phylogenetic clusters (Kudirkiene et al., 2017). A genetic distance matrix, measured in single nucleotide polymorphisms (SNP), was used to detect properties infected with strains belonging to the same clone. Based on previous studies on *S*. Dublin (Mohammed et al., 2016; Ågren et al., 2016) and analysis of differences between same-property isolates in closely-related time periods in the genetic typing data, two isolates were considered as belonging to the same clone, if they had a maximum difference of 15 SNPs between them.

Geospatial data: Coordinates for 114,996 properties were obtained from SEGES in Universal Transverse Mercator (UTM) format, based on Zone 32. The R package 'spTransform' was used to convert this information to the WGS84 system, and the resulting coordinates were joined by property with the movement data.

Businesses definition

Ownership-based definition: Because sequenced isolates, as well as the spatial coordinates, were identified at property level, it was necessary to bring the ownership information to property level. A "primary owner" was selected for properties containing herds belonging to more than one owner. As the objective was to detect as many potentially connected herds

belonging to each business as possible, the primary owners were defined as the ones owning the largest number of herds in different properties. Herds which were assigned to a business were then removed from the list, and a new count of herds by owner was performed, and so on, until reaching only single-owned properties, which were assigned to their sole owner. Ultimately, each business corresponds to one identified primary owner in the dataset. Businesses were created on a yearly basis, to account for ownership changes in herds. Herds changing owner at any part of a year were included in the new business in the next year. Businesses containing properties with sequenced isolates were identified by alphabet letters. Businesses composed of only one property throughout the whole study period were identified by their original CHR number. The remaining businesses were numbered increasingly from "50000001", to avoid confusion with CHR numbers, which contain five or six digits.

Spatial validation and adjustment: The role of the businesses as epidemiological units only makes sense if there is movement of transmission vehicles between the properties and herds within the businesses, such as personnel, machinery or unregistered cattle movement. This is less likely to happen between properties located far away from each other, even if they are connected by ownership. For that reason, after the assignment of each property to a business based on ownership, businesses were validated spatially, based on the distance between member properties. Between-property distances within businesses were calculated and summarized using R packages 'Rgdal', 'psych' and 'geosphere'. Distance matrices (in kilometers) were created with the help of package 'reshape2'. Businesses with internal distances larger than 15 km underwent hierarchical spatial cluster analyses using the package 'fields', to identify the most robust way of subdividing them. The locations of those businesses were also plotted on a map (R package 'sp'), so their distribution in space could be observed. Businesses with properties spread linearly with short distances between them, but with a resulting distance larger than 15 km between the extremities were not subdivided. Sub-businesses were identified by the name of the original ownership-based business, followed by a number. When a business containing one of the properties sampled in the WGS study was subdivided, the sub-business which included that property was always identified by the number one. As an example, properties belonging to the same owner as the sampled property "A" became "Business A". This business was then subdivided spatially in A1, A2 and A3, where the sub-business containing the original property "A" was the one named "A1".

Contact tracing

Movement records were aggregated by month and a variable was created containing the number of animals moved between two properties or businesses in each aggregated period. The final working dataset for properties contained 1,722,978 rows, and for businesses 1,548,751 rows. Both datasets contained variables "source", "destination", "month/year of movement" and "number of animals".

Tracing of contacts was performed using the R-package EpiContactTrace (Nöremark & Widgren, 2014) for each business and property with sequenced isolates. Properties or businesses under investigation were referred to as "roots". An end date (tEnd) was defined for each root, and a tracing period of 3652 days (10 years) was used, based on the interval of isolation of strains of the same clone in the same herds in the data. Each root had a different tEnd, based on the sampling date of strains of the same clone in other properties, in order to find the time when that clone was most likely circulating. The same starting times were used for properties and businesses. Chain length was limited to three steps, therefore containing a maximum of two intermediate units (properties/businesses) between the root unit and the final receiving unit.

From the contact structure file generated by the analysis, all the different pathways from the root unit to the next three units were reconstituted. An "endpoint" was defined as the step of the chain in which a sequenced unit appeared as receiving unit. As an example, if business A moved animals to business 50000001, which then moved animals to business D, D was set as the endpoint, and the maximum distance for this chain was two steps (Fig.1).

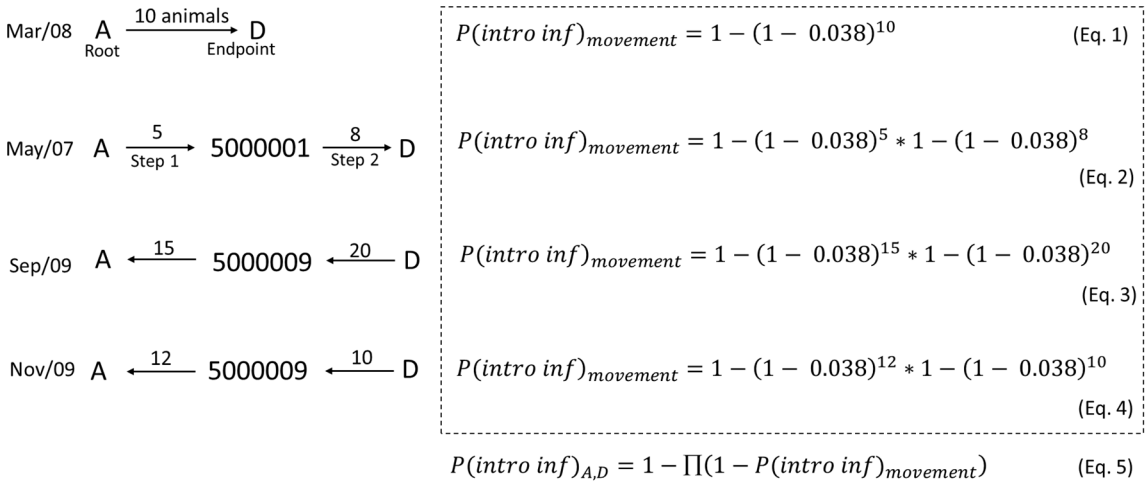

Fig. 1 Simplified illustration of movements through different pathways between a root and an endpoint, and calculation of $P(intro\ inf)_{root,\ endpoint}$.

It is important to note that each step of the chains indicates a movement of a certain number of animals, but these are not necessarily the same animals following all the steps. Earlier in the data management process, large markets, auction houses and cattle dealers were identified by a variable named "Market". This was done because, in principle, it is not possible to affirm that any transmission occurs at a market between two specific units, unless it is the same animals making their way from the root and endpoint units through the market. At the scale this study was conducted, it was not possible to ascertain that this was true for every movement including a market, and therefore, such movements were not included in the remaining of the analysis, as they bring in too much uncertainty. For the remainder of this document, the word "movement" will correspond to the realisation of one pathway between two units at a given time (aggregated movements per month/year).

The impact of the business-based approach on the detection of contacts (i.e. the "effectiveness"), was assessed by comparing the number of endpoints with isolates from the same clone as the root unit between the two approaches.

As mentioned before, an increase in sensitivity in any test or system frequently results in a decrease in specificity. In order to measure the potential gain in detection power against the loss in detection quality, it was necessary to use a quantitative measure to indicate the intensity of contact between a root unit and its endpoints. As the assumed indication of "true contact" between units is the presence of strains of the same clone, it makes sense that the probability of moving infectious animals between two units serves as proxy for the intensity of contact between them. This is based on the fact that, at some point, the strain has been transmitted between two units, and this transmission is more likely to happen when a) the source unit has a high *S*. Dublin prevalence; b) more animals are transferred at each step of the pathway at each movement, c) there are less intermediate pathway steps between two units and d) more

movements occur between two units. This assumption is well-captured by the classic formula for probability of introducing at least one infected animal to another herd, as seen in Eq. (6).

$$P(intro\ inf) = 1 - (1 - p_{\text{source herd}})^n \quad (6)$$

where $p_{source\ herd}$ is the within-herd prevalence of the source herd, and n is the number of animals transferred in a movement.

As the within-herd prevalence for all properties or businesses in the study at all timepoints was not available, an estimate of the average national within-herd prevalence was used for all units. This estimate was based on the winter prevalence for young stock presented in Nielsen (2013). This decision was based on the fact that movements of heifers and calves under one year old corresponded to 77.0% of the dataset, and that winter was the most intensive *Salmonella* shedding time (Nielsen et al., 2011). The chosen prevalence corresponded to 2.3% of faecal culture-positive animals. This was adjusted to a test sensitivity of 60%, as described in the same study, resulting in an estimated within-herd prevalence of infectious animals of 3.8%. This part of the calculations takes care of conditions a) and b) described above, as shown by Eq. (1) in Fig. 1. Because the formula gives the probability of introducing at least one infectious animal for each step of the pathway, the probability for one realisation of a pathway (i.e. one movement) ($P(\text{intro inf})_{movement}$) involving more than two units is obtained by multiplying the probabilities estimated for each step (Fig. 1, Eq. (2), (3) and (4)), therefore reducing the probability in longer pathways, and thus meeting condition c). Condition d) is taken into account by calculating the total probability of introducing at least one infectious animal between a root unit and an endpoint by any possible pathways ($P(\text{intro inf})_{root,endpoint}$), when each pathway may happen several times (as in the third and fourth movements in Fig. 1) throughout the tracing period (Fig. 1, Eq. (5)). Once this calculation is done for each root-endpoint pair detected in the tracing, it is possible to compare its value for businesses which have isolates of the same clone ("true movements"), with the value for non-genetically related businesses. For this approach to be acceptable, the intensity of contact between non-genetically-related businesses should be lower than for between same-clone businesses.

RESULTS

A total of 43,868 businesses were created, with 38,629 (88.1%) being composed of only one property. In all years, between 98.7% (2011) and 99.5% (1998) of businesses were composed of three or less properties. The largest observed business was formed by 15 properties, in 2002. From the 116 businesses created from the 58 properties with *S.* Dublin isolates, 55 (47.4%) were composed of only one property from 1998 to 2017. The largest businesses related to sequenced properties were 'business D1' and 'business LL1', with eight properties each in 2003.

Results of the contact tracing were available for 27 herds and businesses. The remaining 31 units did not have any incoming or outgoing movements of cattle in their tracing periods, or only had movements within a business or through markets. When comparing herds and businesses, the latter consistently detected more same-clone contacts than the tracing between properties. However, they also detected a larger number of contacts involving *S.* Dublin strains that were not related according to the definition used (Table 1), showing that the sought-after increase in sensitivity was attained, but that it was also accompanied by a loss in the quality of the contacts detected. Twelve out of 27 properties had non-related-clone contacts detected, while seven did not have same-clone contacts. Of those, four at least had strains that belonged

to the same cluster of *S.* Dublin strains in Denmark. This adds to the discussion of the correct level of discrimination to define a "clone".

Table 1. Number of contacts of each level of genetic relatedness detected by traceback analyses for properties and businesses.

Unit	Property			Business		
	Same clone	Same cluster	Non-related	Same clone	Same cluster	Non-related
A	6	3	15	8	4	26
AA	0	0	0	5	20	1
B	2	2	4	4	3	5
BB	1	1	0	4	0	1
C	0	1	0	6	19	1
CC	3	4	1	9	9	9
D	7	15	2	8	36	2
DD	1	0	0	3	3	5
E	1	1	1	2	3	6
F	4	5	3	8	4	27
FF	0	0	0	10	15	14
HH	0	2	0	4	11	11
II	10	4	2	16	9	13
KK	2	0	0	6	12	10
LL	2	4	2	7	15	17
NN	7	8	8	8	18	18
OO	7	1	1	8	1	7
P	6	4	0	0	0	0
Q	1	2	0	2	6	4
R	0	0	0	1	15	1
RR	3	9	1	10	26	1
T	0	6	0	0	6	2
U	0	1	0	0	6	3
VZ	1	1	0	8	12	12
W	1	4	0	2	30	1
WW	1	1	0	2	2	5
X	3	6	2	6	15	9

As for the intensity of contact, results show that, in most cases, businesses were more likely to detect genetically-related contacts (i.e., more likely "real contacts"), than non-related ones. Table 2 shows different comparison scenarios, with the probability of introducing (or receiving) at least one infective animal for each root business in relation to endpoints with different levels of genetic relationship. Column "SC/NR" shows the ratio between the probabilities for same-clone and unrelated endpoints, while column "SCC/NR" shows a scenario in which the definition of "clone" has been relaxed to the point where all strains within a cluster are considered the same clone. If all limitations of sampling, testing and business definitions were set aside, the true results should lie somewhere between the two presented ratios. Business "T" had no related contacts, and "P" had no contacts of any kind, so for them it was not possible to do all the calculations.

Table 2. Summarized probabilities of *Salmonella* Dublin bacteria being moved with animals between a root and all its endpoints, according to their genetic relationship using the business concept to link properties.

Business	Mean P(intro inf)$_{root, endpoint}$			Probability ratios	
	Same clone	Same cluster	Non-related	SC/NR	SCC/NR
A	0.67	0.59	0.25	2.7	5.0
AA	0.09	0.19	0.03	2.7	8.1
B	0.93	0.43	0.44	2.1	3.1
BB	0.35	0.00	0.04	8.3	8.3
C	0.50	0.41	0.06	7.9	14.4
CC	0.15	0.05	0.04	3.4	4.6
D	0.75	0.53	0.69	1.1	1.9
DD	0.01	0.01	0.07	0.2	0.3
E	0.97	0.39	0.28	3.5	4.9
F	0.49	0.10	0.23	2.2	2.6
FF	0.61	0.49	0.28	2.2	4.0
HH	0.00	0.04	0.10	0.0	0.5
II	0.24	0.14	0.17	1.4	2.3
KK	0.20	0.02	0.01	20.7	22.5
LL	0.50	0.32	0.20	2.6	4.2
NN	0.39	0.29	0.18	2.1	3.7
OO	0.11	0.01	0.16	0.7	0.7
P	0.00	0.00	0.00	N/A	N/A
Q	0.06	0.08	0.15	0.4	0.9
R	0.95	0.09	<0.01	2245.3	2460.6
RR	0.34	0.20	0.02	20.2	32.3
T	0.00	<0.01	<0.01	N/A	3.3
U	<0.01	0.09	<0.01	0.2	30.9
VZ	0.51	0.24	0.19	2.6	3.9
W	0.65	0.10	<0.01	839.5	967.6
WW	0.30	0.26	0.30	1.0	1.9
X	0.47	0.31	0.24	2.0	3.3

DISCUSSION

The purpose of this study was to provide a proof-of-concept for the use of epidemiological businesses as epidemiological unit for future network analyses. This is becoming increasingly important, due to the structural development of the cattle industry in many countries, characterised by a growing number of large, multi-site cattle production companies.

The principle behind the businesses idea was that the spread of *S*. Dublin between properties within a business is highly likely. Therefore, any introduction of bacteria to one of the properties would, effectively, be equivalent to an introduction to all of them. However, one movement of animals, even from infected herds, does not necessarily result in transmission. The probability of transmission increases with the number of transferred animals, the

prevalence within the source herd, and the number and frequency of animal movements. For links involving other herds/properties between the source and destination, the process of transmission of the pathogen, establishment of said pathogen in the new herd and movements of infectious animals from that herd to the next must happen successfully in all steps of the pathway. Therefore, a link between properties constituted by one movement of one animal going through two other partners before reaching the destination is not as "intense" as a link composed by several movements of several animals transferred directly from the source to the destination.

Phylogenetic information was used to validate the links found between businesses. It was assumed that businesses containing the same clone of *S.* Dublin likely had, at some point in time, movements of infectious animals between them. The animal links found between them by EpiContactTrace, therefore, were considered to make sense. On the other hand, links detected between businesses that did not share the same clone were considered as not necessarily real, and likely to have happened due to the grouping, under the same business, of properties that did not really behave as one epidemiological unit. For that reason, the study evaluated how "intense" the contact between businesses sharing the same clone was, when compared to businesses without non-genetically-related clones of *S*. Dublin. The final pathway probabilities presented for same-clone contacts represent an estimate of the probability that the composition of a business made sense, and the calculation of the ratios between them and the potential artefacts (i.e., non-genetically-related links) should allow us to see how well the businesses concept performed in that sense.

The results indicate that the businesses can potentially be used as the epidemiological unit for trace-back analysis of cattle movements between properties. However, the method is not perfect, and the authors' believe that the quality of the results could be improved by developing more accurate business definitions. Given the number of properties and businesses involved in the current study and the time-period the data was collected from, during which several of the properties changed owner or went out of business, it was not possible to look at each business individually, or collect primary data on relationships between properties. Instead, a generalized classification approach was used. As a result, it is not known if all properties within each business in this study functioned as one unit in real life. It is expected that, in situations involving a smaller number of properties, or in which the researchers can communicate with farmers or managers, the businesses could be individually defined, which would result in more accurate definitions. The study, however, also indicates that this type of information would be highly relevant in an outbreak situation and for future modelling of infectious disease spread within Denmark.

A relatively large number of sequenced isolates were accessed in this study, which became the basis to test if the links found for the businesses made sense. The use of WGS information for such purposes, however, brings in a number of challenges, the first of which is to identify the required level of relatedness between isolates, which characterizes a clone. In fact, clone definition by WGS is an issue of its own; Ågren et al. (2016) describe an *S*. Dublin outbreak in Swedish cattle where the maximum difference between isolates was 13 SNPs. The same paper mentions differences of zero to 26 SNPs among isolates from an endemic region, with no known introduction of new strains. A human outbreak in Ireland found a maximum difference of nine SNPs among isolates, which were all also closely related to a historical isolate, with a maximum 15 SNPs difference from it (Mohammed et al., 2016). This goes back to what was observed by de Knegt at al. (2016), that "relatedness" needs to be associated with the diversity of the gene pool under study and with the application purpose in a way that it is possible to

identify a link between similar isolates, but without so much discrimination that true epidemiological relatedness might be missed. It is also not currently known at which rate *S.* Dublin mutates at nucleotide level over time, or which mutations are important. The differences observed within different types of population by Ågren et al. (2016) and Mohammed et al. (2016) suggest that the levels of differentiation are better defined as population-specific methods, so more sparse samples allow for larger differences, while more locally- and time-related samples allow for smaller differences to be significant. Finally, the clustering process is based on internal differences between sequenced isolates and, therefore, adding a new isolate or changing the reference isolate may have an impact on what the authors' consider a cluster and, ultimately, a clone. In the current study, perhaps some of the same-cluster partners should have been grouped as same-clone.

Another adjustment which could improve the quality of the results would be the definition of an "acceptable" time between pathway steps, which could help remove movements which did not carry any transmission potential. For example, in a pathway with a movement from herd A to B in 2002, followed by a movement from B to C in 2014, the chain could end in 2002. An argument against this would be that *S.* Dublin can persist for many years in the same herd, once it has been introduced. Including serological surveillance data to identify likely persistently infected properties and businesses might have improved the specificity by mainly linking properties with true risk pathways. Another option would be to include a time-based probability-reducing factor in the formulae.

This study utilised an opportunity to use pre-collected isolates from different projects and programmes, combined with register data. However, this means that the sampling process to obtain these isolates was not planned or standardized. Properties could not be randomly selected, and the number of samples per property varied, depending on the original purpose they were collected for. On top of that, only one isolate per cultured sample was preserved to be sequenced. All of this means that the sample of properties was not representative of the region, and that the sample of isolates was not exhaustive of the possible list of *S.* Dublin strains that might have been circulating in the properties they came from. Some of the study properties had more than one circulating strain, so it is reasonable to expect that this was true for some of the others, as well. This affects the assessment of the genetic relationship between properties, which was used as the reference for the quality of contact detection. It is likely that more samples from the same herds would result in more closely-related strains being found for properties which were considered "non-related", implying that the quality of the results here might, in fact, be better than estimated.

All things considered, the results still point in a good direction. In a more controlled study, with fewer properties, more samples/herd and proper metadata, it is likely that a more robust definition of the businesses can be attained. Prospectively, when the definition of businesses is properly adjusted, it can be used to improve the knowledge on the genetic structure of *S.* Dublin populations in Danish cattle, by testing different clone definitions against movement data. The business concept would also be of interest in the investigation of transmission patterns for other diseases, once WGS becomes available on a large pool of isolated strains.

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‘THINKING LIKE A VIRUS’ – THE INFLUENCE OF SOCIAL STRUCTURE AND BEHAVIOURAL CHANGE ON RABIES SPREAD IN DOG POPULATIONS

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SUMMARY

Social structure creates heterogeneity of interactions between individuals, thus influencing patterns of infectious disease spread. GPS data was used to determine the social structure of free-roaming dog populations in three communities in the Torres Strait, Queensland, Australia, and this information was used in a network-based simulation model to predict and characterise rabies spread. Dog networks were ‘small-world’, with characteristic clustering and low average shortest-path length between individuals. Centrality and the duration of contacts between dogs were significantly different between communities. Predicted rabies outbreaks did not propagate in simulated incursions, except when parameters were modified to reflect rabies-induced behavioural changes that disrupt social structure and increase bite probability. Degree centrality was the most influential network parameter on simulated outbreak duration (days) and size (number of dogs infected). It was concluded that the diverse neurological changes induced by rabies infection promote maintenance of rabies in dog populations. Targeted vaccination of dogs with high network centrality could limit outbreak size and duration.

INTRODUCTION

Rabies is a viral zoonosis, estimated to cause 59,000 human deaths annually, >95% of which follow bites from infectious domestic dogs (Hampson et al., 2015). Canine-rabies elimination from endemic regions is challenging (Townsend et al., 2013); it requires integration and coordination of resources, socio-cultural, technical, organisational and political factors, as recently outlined by the World Health Organization, International Organisation for Animal Health, Food and Agriculture Organization of United Nations and Global Alliance for Rabies Control (Fahrion et al., 2017). It is important to ensure that whilst efforts are ongoing to achieve canine-rabies elimination in endemic regions, maintenance of rabies-freedom in neighbouring regions is not be neglected. Oceania is one such rabies-free region. The recent transboundary spread of canine-rabies in south-east Asia (ProMED-mail, 2017) demonstrates that rabies preparedness – strategies and resources to prevent an incursion, ensure early detection of an outbreak and mount an effective elimination response – is essential to protect neighbouring Papua New Guinea (PNG) and Australia.

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The Torres Strait is a region in which biosecurity efforts to protect Australia from a rabies incursion are focused. The Torres Strait lies between mainland Australia (State of Queensland) and Western Province, PNG, and comprises more than 250 islands, of which 18 are inhabited. Travel for traditional purposes and emergency health care between coastal villages in Western Province and the Torres Strait Islands is enabled by the Torres Strait Treaty (http://dfat.gov.au/geo/torres-strait/Pages/the-torres-strait-treaty.aspx, accessed 12.12.17). It is estimated that 30,000 visits occur by PNG residents to Torres Strait communities annually (pers. comm.; Australian Border Force, 2016). Recent studies have demonstrated that travel with dogs between Indonesia and PNG occurs for a range of reasons, often associated with trade, and that the risk of introduction of a rabies-infected dog to Western Province from neighbouring Indonesia is not negligible (Brookes et al., 2017b; Brookes et al., 2018). Although illegal, it is not unreasonable to suppose that domestic dogs occasionally travel between Western Province and the Torres Strait and that a rabies incursion in Western Province would pose a high risk to dogs and people in the Torres Strait (and then potentially mainland Australia).

Owned domestic dogs in Torres Strait communities are generally free-roaming (Brookes et al., 2017a), a type of dog-keeping which is common in many communities in northern Australia (Constable et al., 2010; Burleigh et al., 2015) and elsewhere. Domestic dogs' home ranges can be categorised according to characteristic features and are influenced by an individual dog's sex and reproductive status (Dürr & Ward, 2014; Dürr et al., 2017; Hudson et al., 2017). This likely reflects a complex social structure with heterogeneous interactions between individuals. Contact heterogeneity is known to influence the epidemiology of diseases transmitted by direct contact. For example, Craft et al. (2011) used a disease spread simulation model and showed that the 'small-world' structure of lion prides in the Serengeti made them susceptible to disease epidemics such as canine distemper; Hirsch et al. (2016) demonstrated that seasonal patterns of rabies in raccoons could be explained by changes in social structure. Little is known about the contact heterogeneity in free-roaming dog populations in terms of social structure – for example, who contacts whom and how long is their duration of contact?

Network-agent-based simulation models are able to capture the social structure and contact heterogeneity of the hosts. In the case of rabies, the construction of a realistic network model is not straightforward. Rabies causes encephalitis which induces characteristic behavioural changes that are likely to disrupt the normal social structure, and enhance rabies virus transmission. For example, in a study in Zimbabwe, dogs in the clinical phase of rabies infection were observed to wander outside their usual home ranges (Butler, 1998). Empirical data that describe behaviour changes induced by rabies infection are limited by small numbers of observations in specific environments.

Sensitivity analysis can be used to explore the influence of parameters in disease models. Recently, variance-based global sensitivity analysis (GSA) using the Sobol' method has been used to provide comprehensive assessments of the influence of model inputs. This method accounts for interactions between inputs, as well as non-linear relationships between inputs and outputs (Saltelli & Annoni, 2010; Brookes et al., 2015; Johnstone-Robertson et al., 2017). The use of Sobol' GSA in conjunction with a network-based model of rabies spread provides an opportunity to investigate rabies-induced behavioural changes and their influence on rabies spread in terms of outbreak size and duration.

Here, the objective was to describe networks of associations between dogs on Torres Strait islands using GPS telemetry data. Then, using a simulation model of rabies spread, the influence of network parameters based on these empirical measurements was investigated

relative to other parameters that influence rabies epidemiology – using the Sobol' method for GSA. The results of this study are being used to inform rabies control strategies in the Torres Strait region and also to provide insights on the influence of rabies-induced behavioural changes on rabies spread in domestic dog populations.

MATERIALS AND METHODS

Study site

Three Torres Strait communities on islands with contrasting features were selected for the current study: Warraber, Kubin and Saibai (Fig. 1).

Warraber is a small island community (0.75 km^2; human population 251) surrounded by reef, and is one of four inhabited islands in the Central Group of islands. The terrain is flat, vegetation is coastal heathland and most of the island is easily accessible by people and dogs. Dogs are kept as companions and, at the time of the current study, the estimated dog population was 40.

Kubin is one of two communities on Moa, an island in the Western Group. Moa is a large (171 km^2) volcanic island and vegetation is mixed, comprising dense rainforest areas, as well as eucalypt woodlands and forests. Kubin has a human population of 160 and hunting for pigs with dogs is an important activity on Moa. The Kubin dog population was estimated to be 45 dogs at the time of the current study.

Saibai is one of three inhabited islands in the Top-Western Group. It has a human population of 479 and a geographic area of 104 km^2. Saibai is low-lying, comprising mainly saltmarsh and mangrove vegetation. It is the closest island in the Torres Strait to PNG (3.7km). The Saibai dog population was estimated to include 45 dogs at the time of the current study. Dogs are kept for hunting (deer) and as companions.

GPS units and data collection

The procedures used in this study were approved by the Animal Research Ethics Committee of the University of Sydney (protocol number 2013/6015). Light-weight GPS units (CatLog Gen2, www.mr-lee.com, accessed 20.04.2017) on nylon webbing collars were opportunistically attached to as many dogs as possible within 6-hour time periods at each study site (Warraber: August 2016, Kubin: November 2016, Saibai: March 2017) to achieve maximum GPS monitoring overlap-time between dogs. GPS fix capture-interval was set to 15 s following trials to determine optimal capture-interval to achieve the most accurate estimation of spatio-temporal proximity between pairs of dogs (August 2016; unpublished data). Expected maximum battery longevity was approximately 5 days; therefore, GPS units were collected for analysis after 1 week of deployment at each site.

Dogs were selected during community visits (according to owner availability, due to informed consent requirements). Environmental Health Workers (EHW) and Land and Sea Rangers assisted researchers by seeking permission from owners to allow dogs to participate in the study.

Data analysis

Summary statistics of dog demographics and GPS monitoring (duration, proportion of population monitored and data retrieved) were generated and comparisons were made between study sites. All analyses were performed using the R platform (R Core Team, 2015) and packages 'igraph' (Csardi & Nepusz, 2006) and 'rgexf' (Vega Yon et al., 2015).

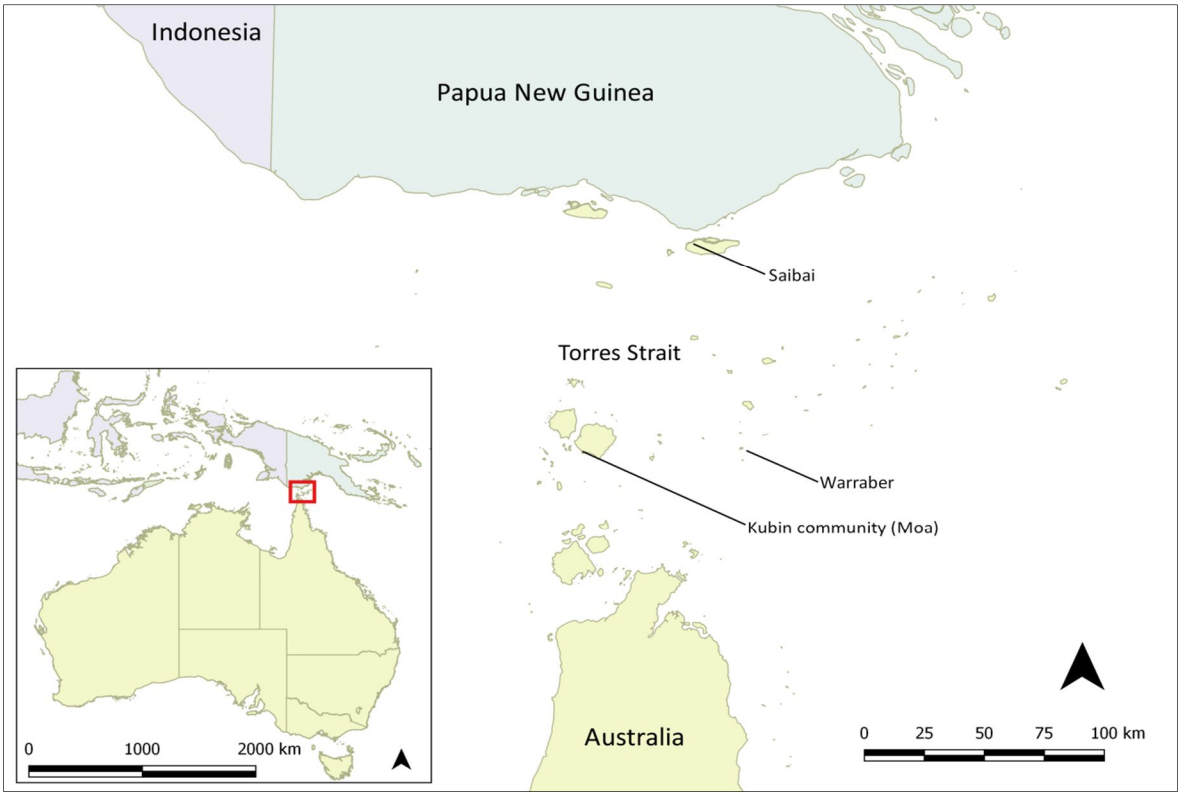

Fig. 1 Map of the study region and sites selected in a study to investigate dog networks using GPS monitoring in communities on islands in the Torres Strait, Australia, in 2016-17.

Network construction and analysis

'Spatio-temporal association' (STa) between two dogs was defined and measured as the proportion of a 24 hour period in which two dogs were within a distance of 5 m in a 30 s temporal window. Matrices of STa were created between all pairs of dogs in each community and used to construct undirected, weighted graphs of contacts for GPS-monitored dogs, in which edge-weights were the STa between pairs of dogs. Networks were plotted and the validity of associations between dogs (expected *vs.* estimated dog pairs and STa) were discussed with each community's EHW.

Network statistics – including network diameter, average path length and global transitivity (clustering coefficient) – were calculated and compared between communities. Summary statistics and distributions of normalised measures of centrality (total degree, Eigenvector and closeness centralities) were also compared. Tie-strength (the sum of STa of individual dogs) was calculated. It should be noted that tie-strength can be > 1 if a dog spends time with more than one other dog simultaneously.

Disease model

A stochastic, agent-based, discrete-time simulation model was constructed and implemented in the Python programming language (www.python.org, accessed 28.04.17) to investigate the influence of social structure and rabies-induced behavioural changes on the duration and number of dogs infected in a rabies outbreak. The model simulated the incursion of a latently infected dog and subsequent transmission to individual susceptible dogs in a single community (population size 50-100 dogs). Effective contacts (contact sufficient for the transmission of rabies following a bite) between dogs were network-based. The course of rabies-infection in a susceptible dog was simulated using an SEIIR process (susceptible [S], latent [E], pre-clinical infectious [I1], clinical [I2] and dead [R]).

Distributions of total degree, edge-weights and transitivity coefficients derived from the current study were used to simulate and parameterise network-associations between dogs in three models (one for each study location). Other model inputs that describe rabies epidemiology in dogs were identical in each model and parameterised from peer-reviewed literature and previously collected field-data (Table 1).

Table 1. Parameter descriptions and values used in a simulation model of rabies spread in a dog population, to determine the influence of social structure on outbreak duration and number of rabies-infected dogs.

Parameter	Description	Values	Data source
Incubation period (days)	Lognormal	μ = 2.76-3.13 δ = 0.54-0.75	Tojinbara et al., 2016
Pre-clinical infectious period (days)	Gamma	Shape = 0.89-3.79 Rate = 0.20-1.16	Fekadu et al., 1982
Clinical period (Cx) (days)	Gamma	Shape = 2.49-3.56 Rate = 0.81-1.19	Hampson et al., 2009
Daily probability of bite given an association (pre-clinical period; dumb form)	Uniform	Range = 0.00693-0.0693	Empirical data (field study 2016; *unpublished*)
Probability of developing furious form	Uniform	Range = 0.2-0.45	Vaughn et al., 1965; Fekadu & Shaddock, 1984; Fekadu, 1988; Foggin, 1988; Jayakumar et al., 1990; Butler, 1998, authors' assumptions
Probability of infection following a bite	Uniform	Range = 0.4-0.52	Hampson et al., 2009, authors' assumptions

Rabies-induced behavioural changes include altered mentation that manifests as confusion or wandering and could increase the duration of association or change the network associations of an infected dog (in both the 'dumb' and 'furious' form of rabies), as well as increased aggression in dogs with the furious form of rabies. These phenomena were parameterised using an increased probability of a bite by a dog affected with the furious form, an increase in STa (edge-weight) to account for increased duration of contact between dogs, and the possibility to 're-wire' a rabies infected-dog's associations to other dogs during each 24 hour period of the

clinical phase of infection. This effectively changed (and overall increased) the infected dog's degree of connectivity to other dogs during the course of infection. Table 2 shows the parameter distributions associated with behavioural change; the daily probability of a bite by a dog with the furious form of rabies was further derived using the distribution of the number of bites by dogs with the furious form of rabies (Table 2) and the duration of the clinical period (Table 1).

Table 2. Parameter descriptions and values used to simulate rabies-induced behavioural changes in a simulation model of rabies spread in a dog population, to determine the influence of social structure on outbreak duration and number of rabies-infected dogs.

Parameter	Description	Values	Data source
Number of dogs bitten during clinical phase (furious form)	Negative binomial	μ = 1.95-2.37, size = 1.23-1.42	Hampson et al., 2009
Proportional increase in edge-weight (STa) during the clinical phase	Uniform	range = 0.2-0.45	Authors' assumptions
Probability of 're-wiring' during the clinical phase	Uniform	range = 0.05-0.25	Authors' assumptions

Models with and without parameters to reflect behavioural changes in rabies-infected dogs were implemented for each community. Outputs from the model included the duration of outbreaks (days) and the cumulative number of rabies-infected dogs during the outbreak. Summary output statistics and distributions were obtained from 10,000 iterations of each model.

The influence of all parameters on model outputs was assessed using Sobol' global sensitivity analysis to determine the total and first-order effect indices (the contribution of a parameter to output variance with and without its interactions with other parameters, respectively).

RESULTS

The highest proportion of the dog population that was monitored and for which data were retrieved was from Kubin community (62 %, n = 24; Table 3). The proportion of collar loss was low in all communities (< 5 %), and the proportion of telemetry datasets retrieved from units was high (> 88 %). The most common reason for inability to retrieve data or for short data duration (Saibai) appeared to be water damage to the GPS units (presumed due to dogs swimming whilst wearing the units).

The overall proportion of monitored female dogs was 46 % (95 % CI 37-54 %) and was not significantly different between communities (X^2 = 0.58, df = 2, P = 0.75; Table 4). The overall proportion of monitored adults was 78 % (95 % CI 66-87 %) and again, was not significantly different between communities (X^2 = 1.18, df = 2, P = 0.55; Table 4). The total monitored population was representative of the proportions of males and females in the source populations for which these data were available (Warraber and Saibai, X^2 = 0.41, df = 1, P = 0.52); information about the age of dogs in the source population was not available.

Table 3. Dog populations, GPS unit monitoring and telemetry data retrieval at three sites (Torres Strait, Australia) in a study to describe dog association networks and their influence on rabies spread, 2016–2017.

	Kubin	Saibai	Warraber
Total dogs	39	43	41
Dogs GPS monitored, n (%)	28 (72)	26 (60)	22 (54)
GPS units retrieved, n (%)	27 (69)	25 (58)	21 (49)
Telemetry datasets retrieved, n (%)	24 (62)	23 (53)	21 (49)

Table 4. Study population demographics, telemetry and network statistics at sites in a study to describe dog association networks and their influence on rabies spread.

	Kubin	Saibai	Warraber
Total dogs (nodes) analysed	24	23	21
Female dogs, n (%)	13 (54)	10 (43)	8 (38)
Adults > 1 year old, n (%)	18 (75)	20 (87)	15 (71)
Median data duration, hours	81.7	51.6	69.0
Range data duration, hours	17.02-123.50	0.13-132.50	2.48-106.60
Total edges	189	110	171
Graph components	1	3	1
Average shortest path length	1.32	1.19	1.19
Network diameter	2	3	3
Median total degree, normalised (95 % range)	0.70 (0.36-0.96)	0.59 (0.03-0.68)	0.9 (0.30-0.95)
Median shortest-path betweenness, normalised (95 % range)	0.01 (0.00-0.05)	0.004 (0.00-0.02)	0.01 (0.00-0.02)
Median Eigenvector centrality (95 % range)	0.76 (0.38-0.99)	0.89 (0.00-1.00)	0.95 (0.33-1.00)
Median closeness centrality (95 % range)	0.77 (0.61-0.96)	0.12 (0.05-0.13)	0.91 (0.57-0.95)
Median edge-weight (STa) (95 % range)	0.0009 0.00-0.41	0.00 0.00-0.30	0.0063 0.00-0.50
Median tie-strength (total STa) (95 % range)	0.39 (0.05-0.91)	0.44 (0.00-1.13)	0.62 (0.01-1.56)
Global transitivity	0.78	0.89	0.89

High global transitivity and low network diameter was a feature of all three networks (Table 4). Figure 2 shows centrality measures for each network. Degree distribution was significantly different between communities (Kruskal-Wallis, $X^2 = 30.9$, df = 2, $P < 0.0001$); dogs in Kubin and Warraber generally had higher normalised degree as well as closeness centralities (> 0.5) than Saibai dogs, reflecting two small network components (1 and 6 dogs each) that were not connected to the main component (16 dogs) in the Saibai network. Betweenness centrality was relatively low (lowest on Saibai) and Eigenvector centrality was high (largest range on Saibai).

Although median tie-strength was similar for dogs in all communities, the distribution of tie-strength between communities was significantly different (Kruskal-Wallis, $X^2 = 97.9$, df = 2, $P < 0.0001$). All measures of centrality, excluding tie-strength, were significantly correlated within communities in this study (Spearman's $\rho > 0.41$, $P < 0.001$). Correlation between degree and the duration monitored was low and not significantly different from $\rho = 0$ (Spearman's $\rho = 0.2$, $P = 0.11$) indicating that increased connectivity (and subsequent centrality) was not dependent on duration of monitoring.

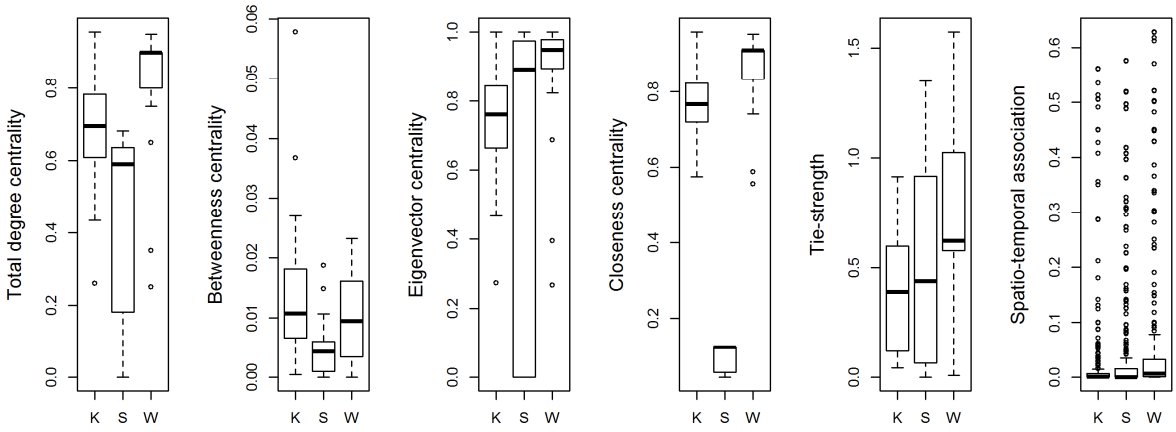

Fig. 2 Boxplots of distributions of normalised measures of centrality of individual dogs and spatio-temporal association between pairs of dogs in three communities in the Torres Strait, Australia, in a study to describe dog association networks and their influence on rabies spread. K = Kubin, S = Saibai, W = Warraber communities.

Median STa was low (Table 4), suggesting that association between at least 50 % of pairs of dogs either did not occur or was fleeting (estimated < 10 minutes/24 hour period). This was apparent in the graphs in which edges were weighted according to STa (an example is shown in Fig. 3 [Saibai]). Once edges with less than median STa were removed, social groups were more apparent (Fig. 3 inset). Discussions with EHWs indicated that the grouping of dogs and their spatio-temporal association derived from the telemetry data reflected their expectations.

Simulated outputs from the rabies-spread models are shown in Table 5; the estimated median duration and number of infected dogs in each community was greater in the models in which degree distribution, edge-weights and the probability of biting in dogs with the furious form were increased. In the models without parameters to reflect behavioural changes in rabies-infected dogs, outbreaks very rarely involved more than 3 dogs (< 5 % of simulated incursions).

Figures 4 and 5 show plots of the Sobol' sensitivity indices (SI) of model parameters for the outbreak duration and number of infected dogs, respectively. Interaction between parameters was important in this model; first-order SIs were relatively low (and uninformative for the purposes of this study) for all parameters' influence on both outbreak duration and the number of infected dogs.

Outbreak duration was most influenced by the duration of the incubation and pre-clinical infectious periods (total SI 0.76 and 0.50, respectively), followed by the size of the dog population (total SI 0.28). Network parameters were then most influential; degree distribution

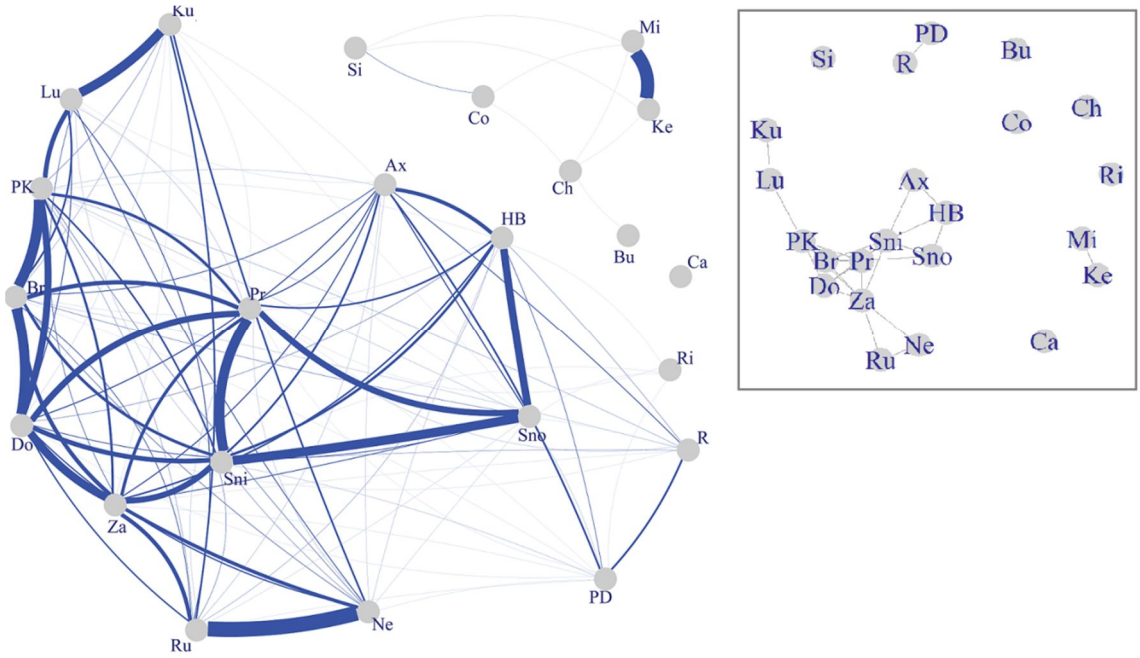

Fig. 3. Graphs of spatio-temporal associations between dogs in Saibai community (Torres Strait, Australia), in a study to describe dog association networks and their influence on rabies spread. Edges are weighted according to duration of spatio-temporal association. Inset = edges > median weight only.

Table 5. Duration and number of infected dogs in simulated outbreaks of rabies in three communities (Torres Strait, Australia) using models with (a) and without (b) parameters accounting for rabies induced behavioural change in a study to describe dog association networks and their influence on rabies spread.

	Duration, days: Median (95 % range)		Infected dogs: Median (95 % range)	
Community	(a)	(b)	(a)	(b)
Kubin	20 (4-118)	55 (4-390)	1 (1-2)	2 (1-66)
Saibai	19 (4-105)	44 (5-323)	1 (1-2)	1 (1-46)
Warraber	24 (5-143)	77 (5-371)	1 (1-3)	3 (1-72)

(total SI 0.20), global transitivity (0.17) and edge-weight (total SI 0.14). The influence of parameters associated with rabies-induced behavioural changes had similar and low individual influence: total SI 0.06 for each of increased bite probability in dogs with the furious form of rabies, increased edge-weight and the probability of re-wiring, but relatively high combined influence (summed total SI 0.18).

The number of infected dogs following an incursion was most influenced by population size (total SI 0.58) followed by degree distribution (total SI 0.35). Although the durations of the phases of rabies infection were still influential (total SI = 0.16-0.31), the network parameters global transitivity (total SI 0.24) and edge-weight (total SI 0.22) were as influential. Overall, network parameters were more influential than increased bite probability in dogs with the furious form of rabies (total SI 0.08), increased edge-weight (total SI 0.06) or the probability of re-wiring (total SI 0.06). However, it should again be noted that the sum of total SIs of parameters associated with rabies-induced behavioural changes was relatively high (summed total SI 0.21).

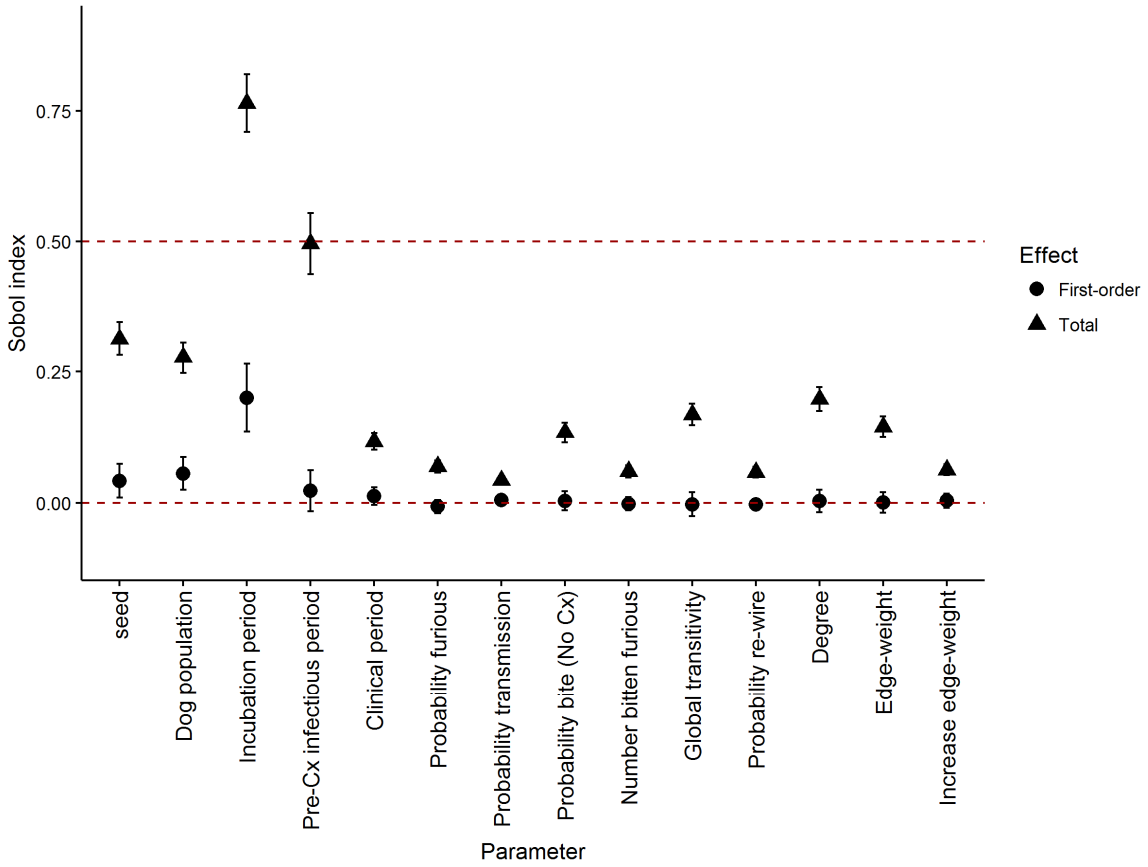

Fig. 4 First-order and total Sobol' indices of parameters' influence on the duration of outbreaks following simulated incursions in a rabies-spread model in a study to describe dog association networks and their influence on rabies spread. Seed = influence of model stochasticity, Cx = clinical signs.

DISCUSSION

The domestic dog social structures described in this study are essentially small-world networks. Originally described by Watts and Strogatz (1998), small-world networks are characterised by local clustering with low average shortest-path length because connections between nodes (edges) are neither completely regular nor completely random. High global transitivity (a high proportion of closed triads indicating clustering) was observed in all three dog populations in this study – in particular Warraber and Saibai – and the average shortest-path lengths were < 2 edges. In terms of infectious disease spread, small-world networks are efficient from the pathogen's perspective; disease can spread rapidly in an epidemic wave amongst locally clustered individuals, as well as simultaneously transmit to other clusters via few individuals (Kuperman & Abramson, 2001; Keeling & Eames, 2005). Despite this phenomenon, it was found that rabies did not propagate to more than one or two other dogs following 95% of simulated incursions in which parameters to describe rabies-induced behavioural changes were not included. In these simulations, transmission depended on the background rate of dog-bites and subsequent probability of infection within a stable social structure with characteristics (transitivity, degree and edge-weight distributions) of the empirical networks. In dogs, rabies virus preferentially infects the limbic system, the thalamus

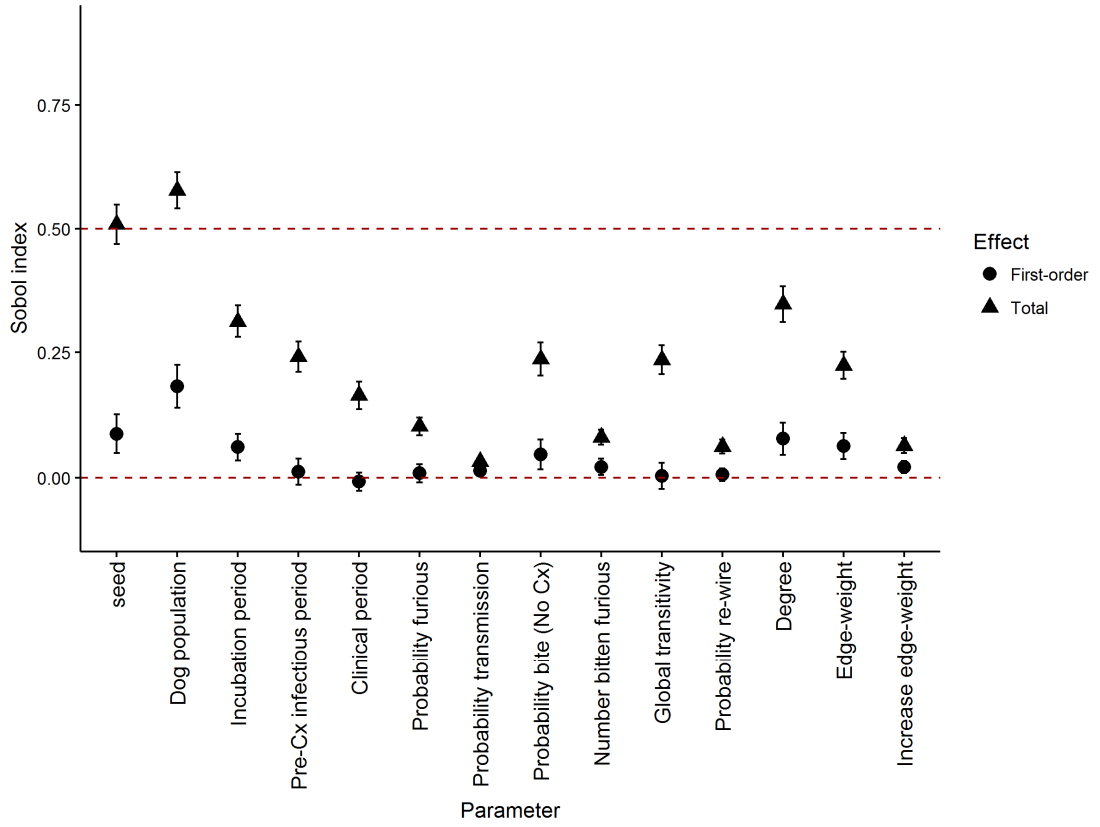

Fig. 5 First-order and total Sobol' indices of parameters' influence on the number of infected dogs following simulated incursions in a rabies-spread model in a study to describe dog association networks and their influence on rabies spread. Seed = influence of model stochasticity, Cx = clinical signs.

and reticular formation of the brain stem, and the trigeminal and vagal nuclei (Suja et al., 2009).

Consequently, neurological clinical signs can be diverse (excitation including aggression ['furious' form], and paralysis ['dumb' form]), and it is assumed that the dominant manifestation of clinical signs depends on the areas of the brain most affected (Fekadu, 1988; Foggin, 1988). When parameters to enhance network connectivity (duration of contact and degree) during the clinical phase of rabies infection and the probability of a bite by a dog with the furious form were included in the model, simulated incursions were longer (combined 95% range 4-390 days [longest in Kubin]) and larger (combined 95% range 1-72 dogs [greatest in Warraber]). Sobol' sensitivity analysis demonstrated that each of these parameters was similarly influential on both duration and the number of dogs infected in outbreaks. The authors' hypothesise that the diversity of neurologically induced clinical signs is an evolutionary feature of rabies virus infection, evolved to maximise disruption of social networks and the opportunity for bites through increased number and duration of contacts, thus increasing the probability of virus transmission.

Sensitivity analysis indicated that, overall, the most influential parameters were incubation and pre-clinical infectious periods and the size of the dog population, followed by parameters that described network structure. Of these, degree centrality was the most influential, particularly on the size of outbreaks. This is consistent with the findings of Christley et al. (2005): an individual's centrality in a network can influence their individual risk of disease and

the subsequent rate of spread and size of outbreaks. This suggests that larger outbreaks could be experienced in communities in which the median degree centrality is high (for example, Warraber), especially if the dog population is large. Dogs with high degree centrality could also act as super-spreaders of rabies virus. Such individuals could manifest as dogs in multi-dog households, dogs with large home ranges or dogs in which rabies induced wandering and aggression.

Vaccination of dog populations can reduce the effective size of the population by reducing the number of susceptible individuals. A rabies incursion in Western Province, PNG, could be an indication for pre-emptive vaccination of dogs in the Torres Strait. However, it is also possible that a rabies outbreak in Papua New Guinea might go unnoticed and that reactive vaccination is implemented following detection of rabies in a Torres Strait community. In this latter scenario, it is suggested that dogs with high degree centrality should be vaccinated first. To support this suggestion, further analysis of the current dataset is required; for example, investigation of associations between degree centrality, home range size and home location of dogs on Warraber, Saibai and Kubin, and modelling to determine the threshold of vaccination required if dogs are vaccinated according to degree centrality.

GPS monitoring of dogs to obtain telemetry data suitable for network analysis is limited by battery longevity of GPS devices and the number of dogs that can be collared concurrently to ensure telemetry data overlap. In this study, network metrics (degree and edge-weight distributions) differed between the three communities. Further analysis is required to assess whether these differences are determined by environmental (island specific) or dog (for example, dog type or purpose) characteristics. Although EHWs confirmed that the networks derived from the telemetry data reflected their knowledge of dog social structure on their islands, it is also possible that social structure differs seasonally or with human activity such as holiday seasons or ceremonies. Finally, the dog populations in this study are relatively small compared to free-roaming dog populations in other northern Australian indigenous communities or in rabies endemic regions. Whilst most empirical networks have small-world structures, methods to obtain network data from larger dog populations and further investigation of the influence of network characteristics in a rabies spread simulation model (for example, the influence of other measures of centrality) are required to generalise the results of this study.

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A STUDY OF CATTLE MOVEMENTS IN NORTHERN IRELAND FOR 2005-2015 USING SOCIAL NETWORK ANALYSIS

E. BROWN*, A.H. MARSHALL, H. MITCHELL AND A. BYRNE

SUMMARY

Animal movements can be represented as a network of herds connected by the trade of animals. Endemic livestock diseases, like Bovine Tuberculosis (bTB) in Northern Ireland (NI), could be facilitated through the networks' structure via the movement of infected animals. Social network analysis assesses movements by calculating network metrics and provides insight into the influence of movements. This method can also be used to evaluate movement restrictions by creating new networks based on changes to the original ones; this study assessed the effect of node removal, either randomly or selectively by considering each nodes' influence. The results suggest that removing nodes based on their influence was more effective than random removal in splitting the networks into many more components. However, the success of targeting nodes was limited due to the connectivity of farms across NI, and suggests that disease control strategies should account for this limitation when being applied to animal movements.

INTRODUCTION

Animal movements have been noted to facilitate long distance spread of disease (Enright & O'Hare, 2016) and are frequently analysed for the purpose of disease prevention and management (El Allaki et al., 2016). In the UK, they have been studied to understand their role in the Foot and Mouth Disease (FMD) outbreak in 2001 (Ortiz-Pelaez et al., 2006) and how they may enable the spread of bTB to susceptible areas (Gilbert et al., 2005). Social network analysis was used to identify the main characteristics of cattle movements in Northern Ireland and then assess how manipulation of such movements could impact the potential spread of infection.

Bovine tuberculosis is an infectious disease, caused by *Mycobacterium bovis*, and is currently endemic in cattle in Northern Ireland (Abernethy et al., 2006). Herd incidence has fluctuated over the last two decades; however there has been a gradual increase in herd breakdowns since 2011 with the herd incidence 12-month moving average at 9.03% in August 2017 (Abernethy et al., 2013; Department of Agriculture Environment and Rural Affairs (DAERA, 2017a). The agriculture industry in Northern Ireland is valued at approximately £1.1 billion per annum, and is detrimentally affected by bTB through decreased animal production, decreased trade and compensation for affected cattle (Allen, 2016; DAERA, 2016). Due to the

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substantial impact that bTB has on the local economy, a control programme has been mandatory since 1964 (Abernethy et al., 2006).

Due to the increase in bTB incidence, its subsequent impact on economic and animal welfare effects, and the imperfect sensitivities of the testing procedures, there is a need for additional research into the management of the disease in support of the long-term goal of eradication. The annual cost of the current bTB management programme rose from £28 million to £34 million between 2015 and 2016; hence the need for mathematical models to understand the mechanics behind bTB incidence levels as these insights may enable more effective use of funds.

Mathematical modelling is advantageous for endemic diseases such as bTB because of the disease's epidemiological complexity that involves multiple host species. Models have been employed in locations such as Great Britain and New Zealand mainly to predict the spatial spread over time (Conlan et al., 2012; Brooks-Pollock et al., 2014; O'Hare et al., 2014). In recent years there has been a focus on using mathematical models to simulate the outcome of various control measures, such as intensive testing regimes and vaccinations (Conlan et al., 2012; Brooks-Pollock et al., 2014). This is beneficial for a range of reasons including ethical considerations, resource restrictions on field studies and the ability to provide region-wide analysis. Models focusing on disease can be informed by risk factors highlighted by field research.

The data used in this study was provided by DAERA from their Animal and Plant Information System (APHIS) and included the variables animal ID, date of movement, ID of previous herd, ID of the next herd and the movement type for all movements between 2005 and 2015. The animal IDs were unique to each individual and remained with them for the entirety of their lives with each registered herd of cattle also receiving its own unique identity number. The type of movement was defined as one of 13 possible types which included special circumstances and births, however the most widely recorded types were (in descending order): standard trade between farms and markets, direct abattoir movements (slaughter), movements into lairage herds, importations and movements for cattle assigned a condemned status.

There were 21,963,941 movements (excluding births which were recorded as movements into their first herd) in the database for 6,154,451 cattle over the 10-year period. The movements were initially considered for each year separately to reduce the computational complexity of dealing with such a large dataset. Although it has been stated that networks created from yearly aggregations of data could be treated only as a close approximation to networks created from daily aggregated data, they have proven to be useful for many epidemiological purposes. Not only did the yearly aggregated data reduce the size of the dataset, it allowed for multiple movements between pairs of herds to be summed and to observe whether the structure of cattle movements had a consistent structure throughout the study period.

MATERIALS AND METHODS

This study used Social Network Analysis, an approach based on graph theory (Diestel, 2006), which is frequently used to describe animal movements to understand disease management (Dubé et al., 2011). This enables insights to be gained on how disease may spread by units who have many interactions, and allows for identification of influential individuals (nodes) (Grear et al., 2014; Hidano et al., 2016). If the structure of the network of individuals

has a temporally consistent pattern, the network itself may be useful for disease management (Vernon & Keeling, 2009). This analysis characterises the network of farms in Northern Ireland by representing farms as nodes that are connected to each other through cattle movements, which are represented as edges.

The network was developed by creating an edgelist of the interacting nodes with direction and the number of interactions between each pair also recorded. The nodes included standard farming herds, markets, abattoirs, and lairage herds due to the large role played by each type of herd in the management of disease. A number of metrics were calculated for each network (Table 1). The network was also assessed for the presence of giant components; subnetworks where all nodes are connected to each other regardless of direction (GWCC; giant weakly connected component). A giant strongly connected component (GSCC) has nodes that are all connected with direction accounted for but may not be present in all directed networks, such as acyclic networks.

Table 1. Network metrics in analysis.

Metric Name	Formula	Description	Relevance	Reference
Degree	$\sum_i E_i$	Number of connections at node *i*.	Analyses the degree distribution.	(Ribeiro-Lima et al., 2015; Gorsich et al., 2016)
Power Law distribution	$P(k) \propto Ck^{-\alpha}$	Nodes with degree *k* can be described by the degree *k* taken to an exponent $-\alpha$ and multiplied by a constant *C*.	Scale-Free networks are best described by this distribution and have a small number of clusters.	(Bigras-Poulin et al., 2006) (Dubé et al., 2011; Ribeiro-Lima et al., 2015)
Network Density	$\frac{\sum_{l=1}^{M} \sum_{i \neq l} E_{i,l}}{N(N-1)}$	*E* represents the edges, *N* is the total number of nodes, *M* does not generally equal *N*	Many networks have low densities due to the connectivity of different nodes	(Dubé et al., 2011)
Average Path Length	$\frac{1}{N(N-1)} \sum_{v_i \neq v_j} \delta(v_i, v_j)$	$\delta(v_i, v_j)$ is the geodesic (shortest path) of nodes v_i and v_j and equals 0 for unconnected nodes.	In directed networks $\delta(v_i, v_j)$ does not necessarily equal $\delta(v_j, v_i)$	(Diestel, 2006; Dubé et al., 2011)
Betweenness Centrality	$\sum_{i \neq j \neq l} \frac{\rho_{i,j}(l)}{\rho_{i\,j}}$	Sums the ratio of the number of geodesics (ρ) through node *l* over all possible paths between nodes *i* and *j*.	Measures node importance with those on more geodesics having a larger weight	(Ribeiro-Lima et al., 2015)
Diameter	$Max_{i,j}\{\rho_{i,j}(l)\}$	Maximum of the set of geodesics for nodes *i* and *j*.	Small diameters (<10) indicate localised movements.	(Gorsich et al., 2016)

Understanding the structure of the cattle trade networks may improve the management of livestock diseases, particularly if the networks' characteristics indicate that the spread of infection is facilitated by cattle movements. If the degree distribution follows a Power Law distribution (Table 1), then the networks could be described as Scale-Free. The networks observed here were compared with a Power Law distribution using the Kolmogorov-Smirnov statistic. Subsequently, comparison was made of the targeted and random manipulation of the networks where nodes were either randomly removed or removed with consideration to their influence in the network. It was anticipated that targeted removal would most effectively fragment the network due to the property of Scale-Free networks, which have been found to be robust to random node removal but vulnerable when influential nodes are targeted (Albert et al., 2000).

The random and targeted manipulation of the observed networks is extremely useful for assessing if a network can be fragmented. A fragmented network could potentially slow the spread of disease or even stop it spreading into some parts of the network. To investigate this, the network can be manipulated using random deletion of nodes, deletion based on the descending order of the nodes' betweenness centrality values, and deletion based on the descending order of the nodes' degree values. Comparing the modified networks using different types of node deletion would assist in further categorising movement networks. Random node deletion has been noted to affect models that have a homogeneous distribution of connections but are not as effective for networks which are noted to have a group of nodes with a relatively high number of connections (Albert et al., 2000). A previous study found that these types of networks also exhibit a GSCC which appears to be easily fragmented using node deletion based on the deletion of nodes with high betweenness centrality or degree values (Albert et al., 2000). These metrics, found in Table 1, were used as indicators of the node's importance to the networks' connectivity, due to their importance in connecting pairs of nodes (betweenness centrality), their higher number of connections (degree), or both. Degree values are especially important as they can be split into in-degree and out-degree so that nodes which would be more likely to move diseased animals to other nodes and those which would have a higher risk of becoming infected can be identified. The GSCC was absent for all the networks in this study due to the acyclic nature of the cattle trade. Because of this, the comparison of node removal methods focused on the fragmentation of the GWCC not the GSCC.

The betweenness centrality and degree values were calculated for yearly networks for 2005-2015 and nodes ranked in descending order according to these values. New networks were created by removing the nodes with the top 1%, 5%, 10%, 20%, 25%, and 50% values for betweenness centrality values and with the same done for degree values. These networks were compared to networks that had 1%, 5%, 10%, 20%, 25%, and 50% of the nodes randomly removed. These node thresholds were chosen to assess if only a small proportion of nodes needed to be removed for changes in network behaviour to be found. All network metrics and manipulations were completed using the 'igraph' package and figures were created using the 'ggplot2' package in RStudio Version 1.0.153.

RESULTS

The number of movements throughout Northern Ireland changed radically from 869,436 movements in 2005 rising to 3,018,340 movements in 2013 at its peak (Fig. 1). Throughout the 10 years, 6,154,451 cattle underwent 21,963,941 movements (excluding births); a mean of 3.57 movements per lifetime, including movements to abattoir. The herds involved included beef

and milk producers, beef breeding, beef rearing, import and export herds, lairage herds and abattoirs.

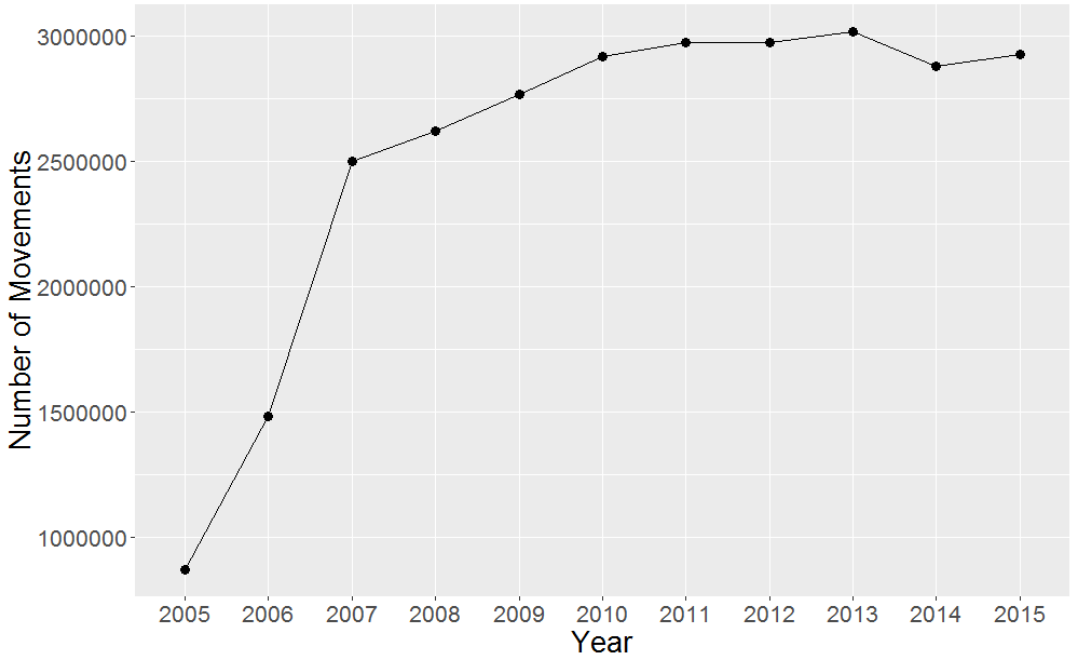

Fig. 1 Frequency of movements for each year 2005-2015

Table 2 displays network characteristics for representative years of the study period. The networks exhibited minor changes in network measures with the density steadily rising over the study period (from 1.38 x 10^{-4} to 2.22 x 10^{-4}), the average path length decreasing (from 4.18 to 3.94), and the mean number of movements occurring between each pair of nodes notably rising (from 6.22 to 18.50). The diameter (longest geodesic) fluctuated between each year (from a minimum 12 in 2008 and 2012 to the maximum of 14 in 2007, 2011, and 2014), however remained constant for the three years included in Table 2. All the networks had non-significant p-values for the Kolmogorov-Smirnov test, and highly significant p-values (<2.2 x 10^{-16}), were produced by the normal, exponential, lognormal and gamma distributions.

Table 2. Network characteristics for the 2005, 2010, and 2015 yearly aggregated networks (given to 3 significant figures).

Network	Density	Average path length	Mean movements between herd pairs	Kolmogorov-Smirnov p-value	Diameter
2005	0.000138	4.18	6.2	0.995	13
2010	0.000220	3.97	18.8	0.977	13
2015	0.000222	3.94	18.5	0.986	13

Table 3 illustrates the component information for the yearly networks. Most of the networks had a small number of components with the largest component (GWCC) consisting of more than 20,000 nodes (approximately 99% of total available nodes). The 2005 network, however, had 18,000 nodes and had 76 components, and hence was very fragmented compared to the other networks which had between 4 and 11 components. Despite this, the largest component of all 10 networks contained more than 99% of the networks' active nodes.

Table 3. Components of the yearly networks for 2005, 2010, and 2015.

Network	Number of components	Size of largest component	Number of operational nodes	Proportion of largest component
2005	76	18591	18747	99.17%
2010	4	23851	23858	99.97%
2015	7	24137	24149	99.95%

The networks for Northern Ireland were found to be acyclic and did not contain a GSCC at any point of the study period. Due to this, manipulation of the networks was assessed by comparing the GWCC of the manipulation networks against the original networks' GWCC.

Figures 2-4 show that the change in average path length, diameter and size of the GWCC, respectively, for the 2010 network when nodes are removed from the network. These changes, however, were not all statistically significant, when assessed by a t-test (using the null hypothesis that the manipulated networks had the same characteristics as the original networks), as the average path length did not significantly change for any of the removal types (betweenness centrality, p=0.1; degree, p=0.1; random, p=0.5). The diameter did significantly change for node removal by betweenness centrality (p=0.04) but not for the degree and random removal type networks (p=0.4 and p=1.00 respectively). The number of components and the size of the largest component in the manipulated networks were statistically different from those characteristics from the network derived from the data. While the networks created using removal by betweenness centrality and degree had increased numbers of components (p=0.02 and p=0.02 respectively, with an alternative hypothesis that the numbers of components were greater than the standard), the networks that had nodes randomly removed did not have a statistically significant change in the number of components (p=0.07). The size of the components was shown to have decreased with all three removal types (betweenness centrality, degree, and random) having significant tests (p=0.03, p=0.03, and p=0.02 respectively).

The average path length, in Fig. 2, increased considerably when the method of removal was by degree, and to a lesser extent by betweenness centrality values, while the method of randomly removing nodes decreased the average path length. The diameter values, shown in Fig. 3, were conflicting as the removal by betweenness centrality was the only one to consistently change the diameter in any direction, which could be due to the definition of the metric. Figure 4 presents the change in the network components with (i) the number of components and (ii) the size of the GWCC. While all three methods consistently increased the number of components, the rate at which they increased was slower with random removal. The same behaviour was observed for the size of the GWCC, with removal by degree and betweenness centrality values reducing the GWCC size faster than through random removal.

The most influential nodes in terms of betweenness centrality were market herds. This was expected since most cattle will traverse the network using market nodes at some point in their lifetime, with the market movements making up 46.7% of the total movements over the 10 years. Nodes with more influence upon the network in terms of degree values were also market herds, with abattoirs and knackeries following closely due to their high in-degree values. This

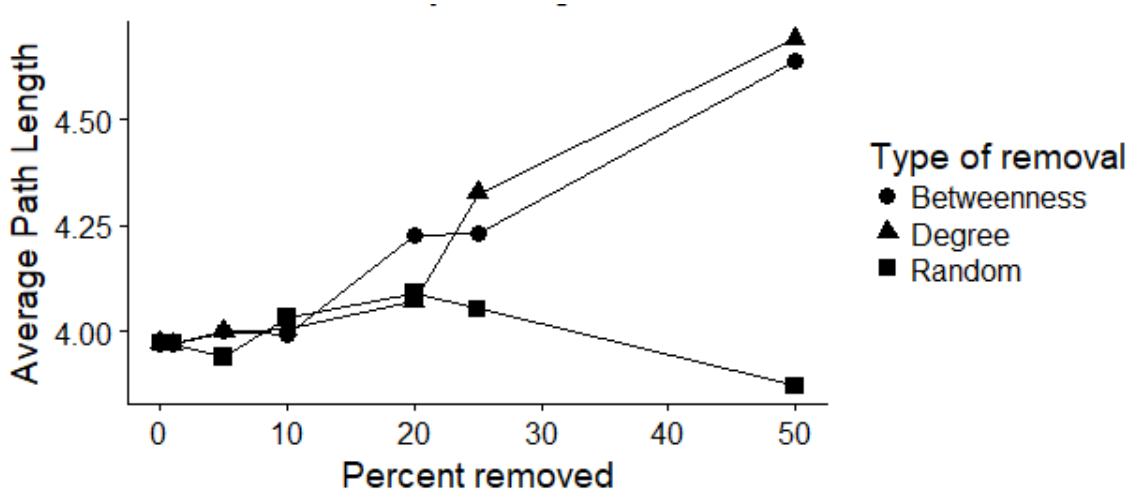

Fig. 2 Comparison of the change in the average path length for the 2010 network as increasing numbers of nodes are removed using three different procedures.

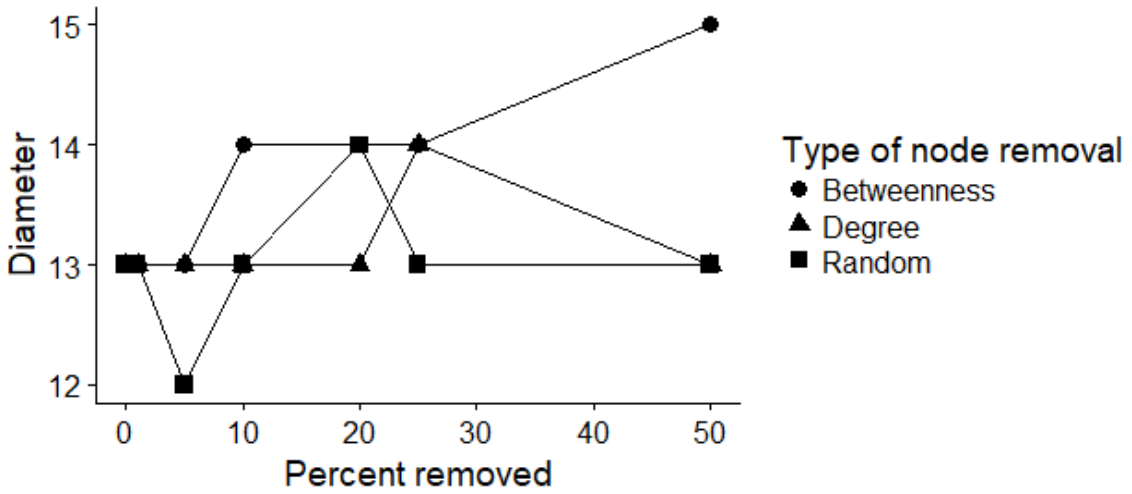

Fig. 3 Comparison of the change in the diameter for the 2010 network as increasing numbers of nodes are removed using three difference procedures

was not surprising given that almost all cattle will end up in either an abattoir or knackery by the end of their lifespan and the combined movement of abattoir and lairage movements make up 13.2% of all movements over the 10 years.

DISCUSSION

Overall, the results found here demonstrated that cattle trade in Northern Ireland is generally characterised by a high frequency of animal movements, resulting in a highly connected and stable network. However, over the ten years of the study, it was shown that there were

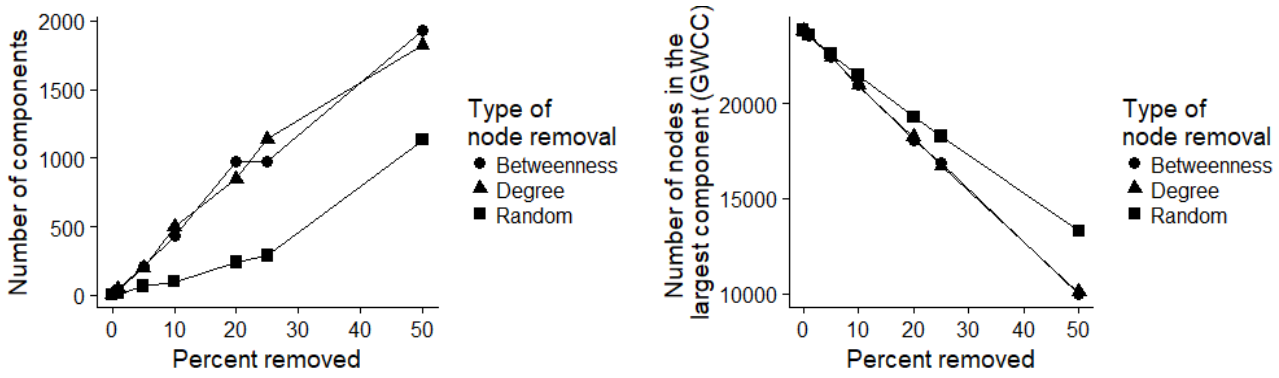

Fig. 4 (i) Comparison of the change in the number of components and (ii) comparison of the change in the size of the GWCC in the 2010 network for three different removal methods.

significant changes in the frequency of such movements. Networks arising from these trade links are characterised by a long-tailed distribution, and generally almost all herds form a single network in any given year. These results have substantial implications for the understanding of animal movements in Northern Ireland and its potential impact on endemic disease spread and maintenance.

The results showed how movements were severely limited in 2005 relative to other years of the study, their frequency rapidly rising thereafter to stabilise with a mean of 2,538,673 movements per year. Statistics on the bTB herd incidence state that the bTB herd incidence was 7.22% in January 2005 (DAERA, 2017b). This resulted in many herds being placed under movement restrictions, which also occurred in 2014 after both herd incidence and movement frequencies peaked at 3,018,340 in 2013. The network metrics did not decrease greatly, and the movement frequencies rose in 2015, which implied that the network is robust when movement restrictions rise for one year.

The number of actively participating farms increased then stabilised to approximately 23,000 farms, but the networks' density steadily increased over time with density equalling 1.38×10^{-4} in 2005 then rising to 2.22×10^{-4} in 2015. This signified that more farms had a larger number of unique connections, suggesting that potentially infected animals could be moved to a wider range of susceptible farms. Along with an increased density, the mean number of movements between each pair of nodes was observed to increase from 6.22 in 2005 to 18.5 in 2015. Not only were there more directly connected node pairs, but the node pairs had become increasingly active which could increase the likelihood that an undetected infected animal was included in the movement. While there were fluctuations in network diameter, the networks included in Table 2 had a constant diameter. This suggests that while there were more unique connections, these connections may stop at another isolated node. Apart from the 2005 network, which had 76 components, all the networks had between 4 and 11 components with the largest component comprising most of the networks' nodes, suggesting that most of the nodes are generally well connected to the wider network.

The networks were assessed for the number of components present and whether the Power-Law distribution would be the best fit to the networks' degree distributions to decide if the networks could be described as Scale-Free. This type of network has been previously shown to be vulnerable to targeted node removals. Since all the p-values for the Kolmogorov-Smirnov

statistic are above 0.05, the Power-Law distribution was the most adequate description of the network's degree distributions.

The modified networks of the original 2010 network created by randomly removing the nodes had a lower average path length, consistent diameter, and increased numbers of components with a smaller GWCC. Those networks created from the removal of nodes based on their betweenness centrality and degree values, however, had larger average path lengths, higher numbers of components and GWCCs with considerably fewer nodes, compared to the randomly manipulated networks. This result was expected since targeting influential nodes would have a greater likelihood of disconnecting neighbouring nodes from the rest of the GWCC. While the greatest difference was at the 50% threshold, this was not a realistic boundary to apply to networks in practice, as it would require that half of all herds in Northern Ireland cease trading. This would have long-lasting economic consequences that would outweigh any benefits brought by the reduction in risk of moving potentially infected animals to susceptible herds.

The hypothesis for this section of the study was that the GWCC would fragment into components with a substantial number of nodes (Albert et al., 2000). This was not reflected in the observations as the number of components did rise but these resulting components contained at most 10 nodes, even when the method of removal was based on high betweenness centrality and degree values. However, node removal based on betweenness centrality and degree values fragmented the network better overall. This was observed in Fig. 4 (i) as the networks with random node removal had 798 fewer components than the networks with betweenness centrality node removal and 692 fewer components than the networks whose nodes were removed according to degree values. Figure 4 (ii) reinforced this idea as the random removal had 13906 more nodes in the largest component than when betweenness centrality was used, and had 13794 more nodes than the largest component when degree values were used.

The structure of cattle movements in Northern Ireland appears to have been affected by movement restrictions due to the yearly herd incidence levels. Lower movement frequencies appear for the period 2005-2009 after the relatively high herd incidence levels prior to 2005, suggestive of the long-term consequences of suspended testing in the 2001 FMD outbreak. Herd incidence spiked again in 2013, with a corresponding drop in movements in 2014 (DAERA, 2017b).

The yearly networks did not show the expected behaviour when increasing numbers of nodes were removed (through either method) and the GWCC remained high even when 20-25% of nodes were removed. This suggests that even if specific nodes were targeted based on how important they were to the network (betweenness centrality) or on the number of unique connections (degree), the GWCC was not fragmented in a manner that would adequately hinder the spread of potential disease across the network. However, removing the nodes based on degree or betweenness centrality increased the average path length indicating that nodes were less connected, thus reducing the risk of infection rapidly spreading through the network. Also, while the targeted removal did not have the desired effect, those types of removal were more successful in creating a small GWCC than random removal.

As has been stated above, the yearly aggregation is an adequate approximation to the aggregation of daily networks, which capture the behaviour of movements more effectively. This study finds that, if disease management strategies are employed, movement restrictions should be focused on identifying the most influential nodes and restricting their movements as this is likely to be more effective in fragmenting the network than random node removal.

However, care must be taken, and analysis of movement data should be undertaken beforehand as the networks in this study have proven to be robust against targeted removal. Thus, diseases such as bTB may need a range of control measures acting simultaneously in combination with strategies such as movement restrictions. This work also highlights the connectivity and robustness of trade networks in Northern Ireland, and how this socio-economic characteristic of farming in the region could be a significant factor negating successful control, containment and eradication of transmissible diseases.

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SURVEILLANCE

EVALUATION OF CLINICAL VETERINARY SURVEILLANCE FOR ANTIMICROBIAL RESISTANCE IN LIVESTOCK

C.P.D. BIRCH*, J.E. PARRY AND P. ALARCON

SUMMARY

Animal health surveillance in England and Wales includes a laboratory-based system relying on voluntary submissions of clinical samples for diagnostic investigation, which includes bacteriological culture of samples, with testing of isolates for antimicrobial resistance (AMR) as appropriate. Sample sizes required to estimate prevalence of AMR, detect trends and demonstrate AMR freedom were calculated. These were compared with AMR data from clinical surveillance in 2015. Data clustering and representativeness were investigated using the 2016 AMR surveillance data. Submissions were sufficiently numerous to estimate prevalence with 5% or 10% precision at 95% confidence for *Escherichia coli* and *Salmonella* in cattle, pigs, sheep and poultry, but not for other pathogens. AMR freedom below 5% prevalence could only be demonstrated for *E. coli* and *Salmonella* in most species, for *Streptococcus uberis* and *Staphylococcus aureus* in cattle, and *Streptococcus suis* in pigs. Pig and poultry submissions were dependent on a small number of veterinary practices.

INTRODUCTION

Emergence of bacterial resistance to antimicrobials critically important for human treatment is of global concern, with a projected 10 M human deaths per year by 2050 (O'Neill, 2014). Livestock may harbour bacterial antimicrobial resistance (AMR), which can also directly complicate efficacy of treatment on farms and the welfare of animals (Woolhouse et al., 2015; Mateus, 2016). Surveillance of AMR is therefore a critical line of defence to monitor and subsequently control this threat. Thus, evaluation of surveillance performance is essential to ensure that new resistance or trends are promptly detected.

Clinical surveillance for antimicrobial resistance (AMR) has been conducted in England and Wales since at least 1971 as part of the veterinary scanning (passive) surveillance system, and has been commissioned by the Veterinary Medicines Directorate (VMD) since 2011. One of the purposes of the testing done under this surveillance is to provide veterinarians in clinical practice with a diagnostic service that helps them investigate disease and provide effective treatment on farms. The testing for antimicrobial susceptibility is funded by VMD, while veterinarians are required only to pay the cost of shipping the samples and isolation of the bacteria.

A further purpose is to "evaluate antibiotic resistance in bacteria of relevance to animal health which have been isolated from diagnostic samples submitted to APHA" (Broadfoot et

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al., 2016). It therefore aims to identify emerging or re-emerging resistances and to monitor trends of existing resistance over time (DEFRA, 2011). It is therefore relevant to determine the sample sizes required to estimate prevalence of AMR and trends over time effectively. Furthermore, surveillance evaluation also requires an understanding of the potential biases that can influence prevalence estimates.

The aims of this study were to:

1. Calculate sample sizes required
    a. To estimate prevalence of AMR at target levels of precision.
    b. To demonstrate that AMR is below a specified prevalence.
    c. To detect changes in prevalence with specified sensitivity.
2. Compare current clinical surveillance submissions with the calculated target sample sizes.
3. Investigate potential sources of bias in clinical surveillance data that may influence or obscure interpretation of AMR prevalence in England and Wales.

MATERIALS AND METHODS

Population represented by the AMR clinical surveillance

The term "livestock" is used here to also include poultry and other birds. Frequencies of AMR from clinical surveillance cannot be extrapolated to the overall livestock population, as only diseased animals with bacterial infection that were submitted to APHA diagnostic services are represented. The frequencies obtained from this clinical surveillance data can be interpreted as prevalence of AMR among a subset of the diseased animal population with bacterial infection in England and Wales, dependent on several assumptions and caveats (Broadfoot et al., 2016). The surveillance includes an unknown proportion of the total number of livestock infections with a given bacterial pathogen. For the purpose of this study, AMR frequencies observed in clinical surveillance are referred to as AMR 'prevalence' in diseased animals.

Sample size calculation

To estimate AMR prevalence: Sample sizes were calculated using an exact method applied in the mathematical software Maple™ 15.01 (Waterloo Maple Inc., 2011). Calculations were based on a Binomial distribution, implying a model of a uniform population in which all individuals have the same probability of being AMR. An observed or observable prevalence will be an integer number of cases x from an integer number of samples n. The notation $B(n,r)$ for the Binomial distribution was used, with n trials and probability per trial r. In this context r may be understood as the unknown actual prevalence, of which x/n is the observed estimate. The notation $CDF[B(n,r),y]$ was used for the cumulative distribution function of $B(n,r)$ up to and including y positive results. The sample size n meets the precision criteria for the sample size required to observe prevalence $r \approx x/n$ if the interval $x/n-z$ to $x/n+z$ is a 95% or higher confidence interval for r, where z is the target precision. Hence the mathematical inequality to solve for this condition is Eq. (1).

$$1\text{-CDF}\left[\mathrm{B}\left(n,\frac{x}{n}-z\right),x-1\right]+\mathrm{CDF}\left[\mathrm{B}\left(n,\frac{x}{n}+z\right),x\right]<0.05 \quad (1)$$

When $x/n-z < 0$ or $x/n+z > 1$, the criteria are adjusted so that 0 to $x/n+z$ or $x/n-z$ to 1 must be a 95% confidence interval. This calculation differs from the widespread method using a Normal approximation to the Binomial distribution (Jacobson, 1998). It takes account of the discrete nature of estimating prevalence from an integer number of cases among an integer number of samples, with the result that the minimum sample size will often be sufficient to exceed the target precision; it also estimates precision as a symmetric interval around the estimated prevalence $r \approx x/n$. The most important benefit is that the exact calculation estimates valid sample sizes across the whole range of prevalence from 0.0 to 1.0, except it can only estimate sample sizes for rational values of prevalence.

These calculations aimed to achieve targets for precision, but it may be noted that detection of AMR with sensitivity or specificity below 100% would reduce the accuracy of estimated AMR prevalence. Thus Eq. (1) would be valid for comparing two different measures of AMR prevalence with the same imperfect sensitivity and specificity, but the accuracy of the measures would be lower than their precision.

The prevalence of AMR is uncertain before sampling, so sample sizes required for a target precision at 50% prevalence may be used to guarantee samples are large enough. However, antimicrobial resistance occurs at different prevalence levels in the population, and the assumption of 50% prevalence will substantially overestimate sample size for most cases. The sample sizes from Eq. (1) could be reduced if the sample was a large proportion of its background population, i.e. the sample was expected to be more than about 5% of the population it represented (Barnett, 2002), but it was assumed this did not apply. Sample sizes were calculated for different levels of prevalence, and levels of precision = 2%, 5%, 10% and 15%, with 95% confidence. To evaluate current submission levels, the number of isolates tested for different bacteria in each livestock species, and the AMR prevalence observed, as shown in the UK Veterinary Antimicrobial Resistance and Sales Surveillance (VARSS) report 2015 (Broadfoot et al., 2016), were plotted against the calculated sample sizes.

To demonstrate freedom from AMR: Calculating the number of AMR free samples required to demonstrate that prevalence is below a specified level is a special case of the exact calculation of Eq. (1), where $x = 0$ as in Eq. (2).

$$\begin{aligned}&\mathrm{CDF}[\mathrm{B}(n,z),0]<0.05\\ &\Rightarrow\ (1-z)^n<0.05\end{aligned} \quad (2)$$

Hence the method for calculating sample sizes for demonstrating freedom from AMR (or for setting given upper limits on the prevalence of undetected AMR) is consistent with the method for calculating sample sizes at non-zero prevalence. The solution of Eq. (2) matches the long established formula used to calculate sample sizes required to demonstrate freedom from infection (Martin et al., 1992). The resulting target sample sizes were then compared with the number of isolates submitted in 2015 for each type of bacteria and livestock species, extracted from the VARSS report 2015.

To detect change in AMR prevalence: The proposed standard for determining changes in AMR prevalence was 80% power to detect a difference significant at a 95% confidence level (The EFSA Journal, 2007). For non-zero initial prevalence, the Piface calculator developed by Lenth (2011) was used. This calculator uses a Normal approximation approach, with a continuity correction to increase sample sizes to take account of the discontinuous nature of

the Binomial distribution. Results were generated assuming that both samples would have equal sizes. Piface will not allow selection of combinations of sample size and prevalence that generate less than 5 positives or negatives, which prevented calculation of sample sizes for some combinations of initial prevalence and change.

For increases from zero prevalence, a simple logic based on the Poisson distribution was applied. At low prevalence, the Binomial distribution is approximated by the Poisson distribution, which only has a single parameter, the expected number of cases λ, which is also the variance. The Poisson distribution can itself be approximated by the Normal distribution. Hence, by analogy with the Normal distribution, the expected number of cases should be 2.802 standard errors from zero to meet the required standard of 80% power to detect a difference significant at a 95% confidence level. This generates the formula for Eq. (3).

$$2.802\sqrt{\lambda} < \lambda \Rightarrow \lambda > 7.851 \quad (3)$$

Hence, the minimum sample size > 7.851/prevalence.

Evaluation of current selection bias and confounders in the clinical surveillance data

To understand the representativeness of the clinical surveillance data, factors related to selection bias or confounding bias were explored. Confounding factors represent those data features (or sub-sets of the data or population) across which prevalence measures need to be balanced in order to make them comparable, as AMR prevalence would be expected to be different or not comparable. Factors related to selection bias (data clustering) indicate variables where samples are unevenly represented due to repeated observations (e.g. more than one submission per farm or disease incident) or gaps in coverage of all parts of a population.

In particular, repeated submissions by farms and senders were analysed in the 2016 AMR clinical surveillance data for the UK. The county parish holding (CPH) number (a farm identifier) and postcodes available in the data were used to estimate the number of farms represented in the surveillance. The name of the sender was used to estimate the number of different senders submitting samples to the APHA AMR diagnostic services. Senders could represent a veterinary practice or a farm company, which might submit samples from multiple farm locations. The population of holdings, as from Livestock Demographic Data Group reports produced by APHA, were used to calculate the probability of a farm submitting samples to this surveillance. In addition, Lorenz curves were produced and Gini coefficients were calculated to measure how unevenly samples were distributed among farms and senders (Gini, 2005).

RESULTS

Sample sizes to estimate AMR prevalence and comparison with 2015 AMR surveillance results

Examples of sample sizes to estimate AMR prevalence at precisions 2, 5, 10 and 15% with 95% confidence, assuming random sampling, are shown in Table 1. When comparing sample size estimates with number of isolate submissions and AMR prevalence observed in 2015, the results show that all the 46 AMR prevalence observations from *E. coli*/coliform isolates had sufficient sample size for a 10% precision in their estimation (Fig. 1). Of these, 29 (63%) AMR observations had sufficient sample size to allow a 5% precision, including all observations from cattle. On the other hand, the sample size for the majority of prevalence estimated from sheep

and pigs only allowed a 10% precision. Nonetheless, even in these species, the sample sizes were sufficient for 5% precision when observing AMR with low prevalence.

Table 1. Sample size required to estimate 'prevalence' of AMR at precisions 2, 5, 10 and 15% with 95% confidence, dependent on prevalence and target precision.

	Precision			
Prevalence[a] (%)	2%	5%	10%	15%
0	149	59	29	19
1	212	73	35	23
5	516	105	40	23
10	918	160	50	28
15	1300	216	60	30
20	1585	267	72	34
30	2066	339	89	42
40	2353	388	101	45
50	2449	402	104	47

[a]Prevalence is approximate, because sample sizes can only be estimated for observable values, i.e. ratios of integer cases (including 0) from integer sample sizes.

Similar graphs to Fig. 1 were used to investigate AMR among other pathogenic bacteria. Sample sizes for 75 types of AMR prevalence observed from *Salmonella* isolates were sufficient for a 10% precision in their estimation. Sample sizes for cattle, chickens and the majority of turkey AMR testing were sufficient for a 5% precision in the prevalence estimates. The sample size for the majority of AMR *Salmonella* testing done in sheep and pigs only allowed a 10% precision estimate.

Sample sizes for other types of isolates tended to be smaller. Three bacteria isolated from mastitis in cattle, *E. coli*, *Streptococcus uberis* and the majority of *Staphylococcus aureus* had large enough sample sizes for a 10% precision. Samples for a few other bacteria, such as *Streptococcus suis* in pigs, were sufficient for 15% precision, but sample sizes of other pathogens were too small to measure prevalence with any confidence.

Sample sizes to demonstrate AMR prevalence in a population is below specified levels

Table 2 shows sample sizes required to demonstrate AMR prevalence is below 1, 2, 3, 5, 10 and 15% at 90, 95 and 99% confidence. For comparison with Fig. 1, note that samples of 149, 59, 29 and 19 are sufficient to potentially demonstrate AMR prevalence below 2, 5, 10 and 15% at 95% confidence. These numbers match the values on the curves in Fig. 1 at zero prevalence, demonstrating the consistency between the sample size calculations at zero and non-zero prevalence.

Observed sample sizes for *E. coli* coliforms and *Salmonella* in cattle, chickens, pigs and sheep, *E. coli* associated with mastitis, *S. uberis* and *S. aureus* in cattle and *S. suis* in pigs were large enough to conclude unobserved AMR were below 5% prevalence with 95% confidence. AMR absent from samples for *Pasteurella multocida* and *Streptococcus dysgalactiae* in cattle and *Bibersteinia trehalosi* and *Mannheimia haemolytica* in sheep could be interpreted as below 10% prevalence, while there were also sufficient isolates of *M. haemolytica* in cattle and *Actinobacillus pleuropneumoniae* in pigs to demonstrate AMR prevalence below 15%. Other samples were not large enough to reach any conclusions about the presence or absence of AMR.

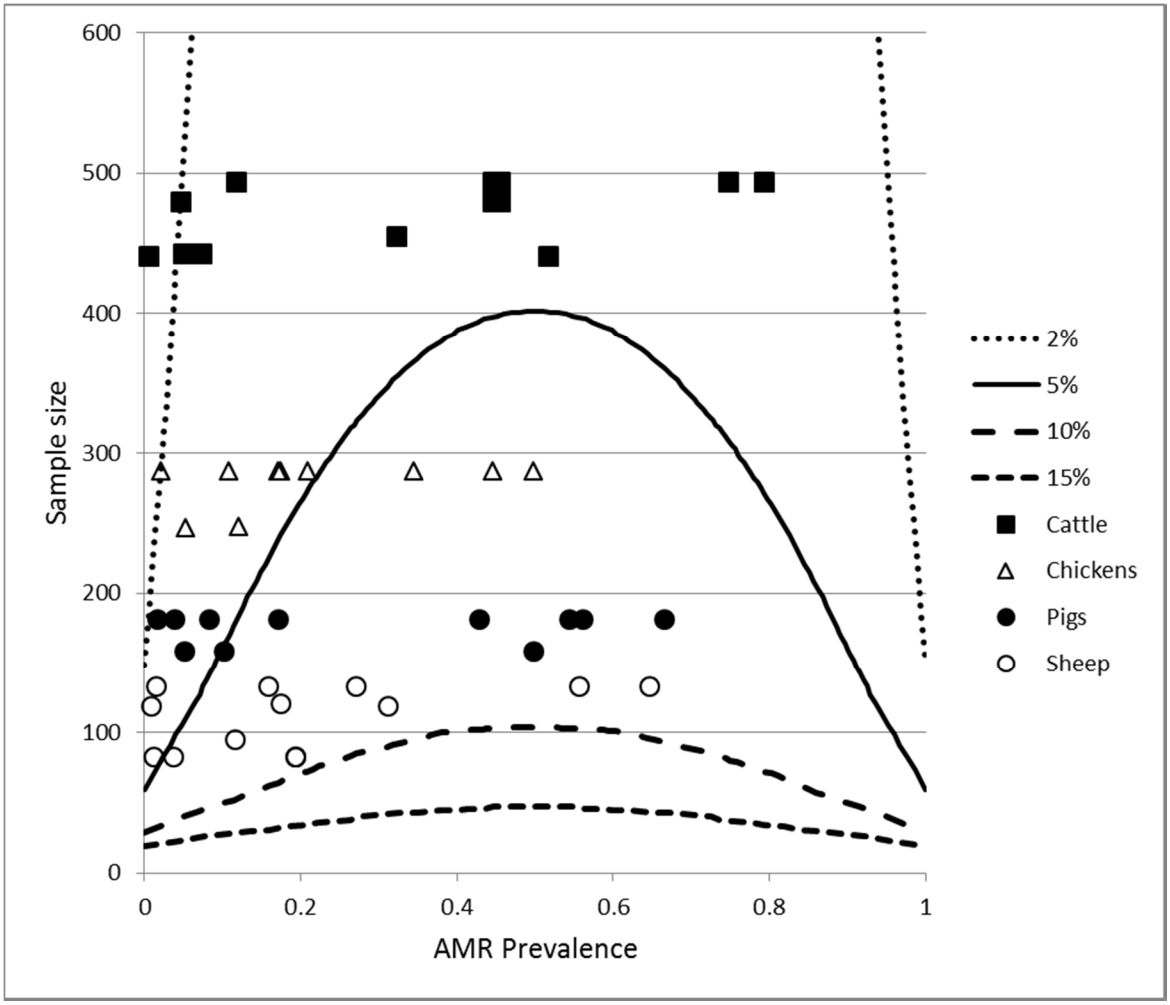

Fig. 1 Lines indicate sample sizes required to observe AMR prevalence at target precisions of 2, 5, 10 and 15% at 95% confidence. Points indicate prevalence of various AMR investigated in four livestock species, with actual sample sizes (Broadfoot et al., 2016).

Table 2. Sample size required to demonstrate unobserved AMR is below various levels of prevalence at 90, 95 and 99% confidence.

	To show resistance is below					
Confidence level	1%	2%	3%	5%	10%	15%
90%	230	114	76	45	22	15
95%	299	149	99	59	29	19
99%	459	228	152	90	44	29

Sample sizes to detect a change in AMR prevalence

Table 3 shows sample sizes to detect increases of AMR prevalence from an initial prevalence up to 50%. The table is reversible, e.g. a sample size of 49 to detect an increase of 30% from 40% implies that the same sample size would have the same power to detect a decrease of 30% from 70%. Smaller changes can be detected starting from an initial prevalence of zero: a sample of 786 to detect an increase from 0 to 1%, 393 for 0 to 2%, 262 for 0 to 3% and 197 for 0 to 4%.

Table 3. Given baseline prevalence up to 50%, the sample size required for 80% power to detect various amounts of increase of AMR prevalence at 95% confidence.

Prevalence	+5%	+10%	+15%	+20%	+25%	+30%	+35%	+40%
50%	1605	408	183	103	66	45	33	-[a]
40%	1573	408	186	107	70	49	36	28
30%	1416	376	176	103	68	49	37	29
20%	1134	313	151	91	62	45	35	28
10%	725	219	113	72	51	-	-	-
0	158	79	53	40	32	27	23	20

[a]The Normal approximation is invalid when the number of AMR or non AMR in the first or second sample < 5.

Analysis of confounding and selection bias factors

Various levels and sources of data clustering can allow potential selection bias, but this paper only reports on analysis of clustering associated with multiple submissions by farms and veterinary practices. Only small proportions of all livestock farms made at least one clinical submission included in the AMR data for 2016, being 3.1% of pig holdings, 1.4% of cattle holdings and less than 1% of holdings for other species. The average numbers of submissions per farm that made a submission were higher than would be expected if submissions came from random selections of holdings, being about 1.9 for chickens, 1.4 for pigs, 1.23 for cattle and 1.08 for sheep. However, these ratios suggested that, apart from chicken submissions, most submissions were the first submissions in the year from the farms they came from, limiting the potential for selection bias.

Clustering by sender (veterinary practice) was more of an issue. Veterinary practitioners vary widely in their use of APHA diagnostic services, due to a range of factors such as market prices and the related financial performance of farm clients, proximity to or relationship with an APHA laboratory, degree of specialisation or accessibility of an alternative private laboratory. Some veterinary practices or companies cover large numbers of farms, so they could substantially impact the selection of farms represented in the surveillance (selection bias). Submissions per sender making any submissions were 9.8 for chickens, 6.3 for pigs, 4.5 for cattle and 2.9 for sheep. Moreover, the Lorenz curves and Gini coefficients suggested that the submissions were unevenly distributed among senders. Whereas the highest Gini coefficient for distribution of submissions among farms was 0.41 for chickens, the coefficients for distribution among senders were 0.79 for chickens, 0.72 for pigs, 0.52 for cattle and 0.46 for sheep. For pigs, only 13 senders (21%) accounted for over 81% of submissions. For chickens, one sender accounted for over 53% of submissions and 7 senders accounted for over 92% of submissions.

DISCUSSION

This is a first review of sampling under the AMR clinical surveillance in England and Wales. Given various caveats, current numbers of samples would be sufficient for estimation of prevalence to at least 10% precision at 95% confidence for isolates from *E. coli*, coliforms and *Salmonella*. However, increased submissions from pig and sheep are needed to estimate prevalence to 5% precision with 95% confidence, which might be considered a minimum standard for timely detection of changes in AMR. The caveats include acknowledgement that representative sampling of disease incidents presents a number of challenges, some of which

are discussed here. Individual *Salmonella* serovars were not considered separately in the analysis, although different serovars may have characteristic associated patterns of resistance. The numbers of *E. coli*, *S. uberis* and *S. aureus* isolates obtained from mastitis in cattle were also large enough to achieve 10% precision. For most other pathogens, the sample size did not allow an accurate estimate of prevalence, particularly when prevalence was not very low. The sample size calculations provided in this study could help to decide the minimum sample size requirement for different types of AMR in specific isolates. Combining data from more than one year could increase sample size, but would erode temporal resolution. Combining years until target sample sizes are achieved could also introduce bias, because smaller sample sizes may seem sufficient when low prevalence of AMR is found.

Several limitations on the interpretation of AMR prevalence should be emphasized. The samples submitted that contribute to clinical AMR surveillance correspond to a subset of the diseased livestock population suspected of bacterial infection. By definition submissions to clinical surveillance are dependent on a range of factors, including the prevalence of diseases on farms and on the capacity of farmers and veterinarians to identify these and to use the APHA diagnostic service. It is possible that many bacterial infections are treated without having an AMR diagnostic test performed by APHA, so submissions in this surveillance may mainly represent situations where a disease outbreak is severe or treatments have been ineffective. Further information on the reasons for submissions is needed to clearly understand the population represented in this surveillance and whether it changes from year to year.

The levels of clustering for some livestock species are also an important factor to consider. The calculations for sample size in this study assume that the samples are uniformly comparable with each other. Estimates based on the Binomial distribution can be expected to be robust to deviations from uniformity so long as samples are random. In particular, the probability that a bacterial isolate is sampled should be independent of the probability that it is resistant. Sampling is biased if the probability of being sampled is positively or negatively correlated with the probability of being AMR. Clearly, the reasons for making diagnostic submissions for clinical investigation are potentially related to the presence of AMR, so bias from this source is likely. Various confounding factors and clustering may considerably impact prevalence estimates, requiring cautious interpretation of the observations, or, if possible, clarification by additional analysis or sampling. Issues include the testing of individual isolates for multiple AMR, the generation of multiple isolates from samples, and multiple samples, often of different types, from submissions. The age of diseased animals, the production system, geographic location and season are examples of further factors that may influence the AMR detected. Accounting for such issues may require an increase in the sample size estimates provided here. Analysis of clustering just from multiple submissions by farms and veterinary practices identified issues, including dependency of the pig and poultry submissions on a relatively small number of veterinary practices.

Arguably the precise calculation of sample sizes required to achieve target levels of precision or power is not critically important. Some recent guidance still presents sample sizes to meet precision targets for proportions, such as prevalence, calculated by the simple but obsolete method of Normal approximation to the Binomial distribution (Jacobson, 1998; OIE, 2013). However, this method is unsuitable for estimating levels of AMR unless at least 30 resistant and 30 non-resistant samples are identified (Barnett, 2002). Moreover, uncritical application of methods like the Normal approximation at low prevalence can generate recommendations that are clearly incorrect. As an illustration, the Normal approximation would suggest that a sample of 96 is enough to estimate prevalence at 1% with precision 2% (Jacobson, 1998; The EFSA Journal, 2007, Annex 1); the more plausible sample size

recommended here for the same purpose is 212 (Table 1). The availability of computing resource and applications to assist statistical calculations should make high statistical standards more readily achievable than in the past. Therefore, it seems timely to review optimal statistical approaches and appropriate sample sizes. Potentially, exact calculation or Bayesian estimation could also be applied to the estimation of sample sizes to detect trends in AMR over time.

Clinical surveillance provides a simple and cost effective method to review long term trends in resistance (individual and multi-drug) in a wide range of bacterial isolates recovered from food producing animals using a standardised panel of antibiotics, including those critically important in human medicine. It provides evidence for treatment purposes and may be a driver of appropriate prescribing for current and future clinical cases. Resistance profiles are also useful as an epidemiological marker, for example pentavalent (ACSSuT) resistance in *Salmonella* Typhimurium DT104 and the advent of whole genome sequencing greatly increases the ability to identify and understand mechanisms of phenotypic resistance. There is evidence of early detection of emerging resistance threats, recently demonstrated by the detection of transferable colistin resistance at a low estimated prevalence (Anjum et al., 2016; Duggett et al., 2017). The monitoring of AMR in bacteria from clinical veterinary diagnostic submissions is complemented by statutory EU monitoring, which comprises harmonised targeted monitoring of AMR in zoonotic and commensal bacteria in healthy animals at slaughter (Decision 2013/652/EU).

Finally, other potential methodologies, such as scenario tree modelling (Martin et al., 2007), may also be considered for the evaluation of the effectiveness of clinical AMR surveillance. Furthermore, additional epidemiological analysis within the context of the wider objectives of scanning surveillance is recommended to assess for risk factors associated with submission of samples to surveillance, to determine trends of submissions over time, and to extend statistical analysis of clustering data. It is likely that future clinical AMR surveillance design will be complemented by developments in human, food and environment surveillance, which could help to refine the accuracy of the existing surveillance in livestock (Queenan et al., 2016). Future similar studies but directed to evaluate efficacy of One Health surveillance to detect associations and changes in pattern of AMR between humans and livestock would be necessary.

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HOW TO APPLY A ONE HEALTH APPROACH TO SURVEILLANCE: A SYSTEMATIC LITERATURE REVIEW

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SUMMARY

One Health surveillance is intuitively appealing and suggests advantages regarding performance and cost-effectiveness, when compared to more conventional approaches. Nevertheless, a conceptual and methodological framework is missing to (i) characterise what a One Health surveillance system is, (ii) define appropriate levels of inter-sectoral and multi-disciplinary collaborations depending on the surveillance objective and context. A systematic literature review of existing One Health surveillance systems was conducted to define the organisational and functional characteristics of such systems. The analysis of the 41 systems retrieved led to the identification of different modalities of collaboration in terms of governance and operations as well as to the description of influential factors that favour or hamper their implementation. The findings of the study underlines that a conceptual framework is missing to define exactly the notion of One Health surveillance and this hampers the operationalisation of collaborative efforts for efficient and sustainable surveillance systems.

INTRODUCTION

Nowadays there is international consensus that highlights the necessity to develop integrated and systemic policies to efficiently manage health issues at the human-animal-environment interface. This is in line with the One Health (OH) concept, which promotes collaborative efforts across sectors and disciplines to attain optimal health for humans, animals and ecosystems (Zinsstag et al., 2011). Close collaborations between health systems are therefore strongly encouraged (FAO, 2010), particularly in the field of surveillance of health hazards involving humans, animals and their environment.

Surveillance is the systematic and ongoing collection, validation, analysis and interpretation of health data to disseminate information supporting decisions for appropriate heath interventions (Choi, 2012). Nowadays, there is no consensual definition for a OH surveillance system. Stärk et al. (2015) and Berezowski et al. (2015) characterise a OH surveillance as a system collecting data in multiple domains. For Hattendorf et al. (2017), using a OH approach for surveillance does not automatically imply that the surveillance system must collect data from animals and humans. To define OH surveillance, Karimuribo et al. (2012) emphasize the

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need for collaborative efforts between the human and animal (wildlife and domestic) sectors in the surveillance process.

This concept is intuitively appealing and suggests advantages regarding performance and cost-effectiveness of OH surveillance, when compared to single sectoral approaches (Zinsstag et al., 2011). Politicians and international organisations have embraced the concept and are strongly advocating for more collaborations across sectors for the surveillance of zoonotic diseases and other health hazards at the human-animal-environment interface (FAO, 2010). Internationally and nationally, policy-makers are also pushing in this way. Nevertheless, they are still few operational and sustainable OH surveillance systems (Häsler et al., 2014; Stärk et al., 2015).

The difficulty of operationalising OH in the context of surveillance may come from a lack of conceptual and methodological framework to support the identification and the implementation of appropriate inter-sectoral and multi-disciplinary collaborations to ensure a performant surveillance system. Collaborations might be implemented at various degrees and different stages of the surveillance process, depending on the surveillance objective and context. The purpose of this review is therefore to identify and analyse the existing OH surveillance systems to define their organisational and functional characteristics, the context in which they are implemented, as well as the influential factors which may obstruct or support their implementation and performance.

MATERIALS AND METHODS

In absence of consensual definition for a OH surveillance system and based on elements found in published literature (Karimuribo et al. 2012; Berezowski et al. 2015; Stark et al, 2015; Hattendorf et al., 2017), the following definition is proposed for the purpose of this study. A OH surveillance system is a system in which collaborative efforts exist across at least two sectors (among human health, animal health, food safety and environment) in the surveillance process to produce and disseminate information with a purpose to improve any of human, animal or environmental health.

Literature sources and search strategy

A systematic literature search was conducted according to the PRISMA requirements (Preferred Reporting Items for Systematic Reviews and Meta-analysis) (Moher et al., 2009). Searches were conducted in Google Scholar, PubMed and ScienceDirect. The literature search focused on scientific and grey literature in French and English published between 01/01/1985 and 31/12/2016. Four different domains were used and applied on title, abstract and key words only: Surveillance, OH approach, Health hazards, Domains.

Study selection

All documents retrieved in the bibliographic databases were screened by two reviewers following two distinct steps. For the first step three inclusion criteria were applied on titles, abstracts and keywords: (i) the document describes a surveillance system (as defined previously), (ii) the surveillance system focuses on a health hazard, (iii) the surveillance system shows evidence of collaborative efforts among professionals working in at least two different sectors, among animal health, human health, plant health, food safety and environment. In the second step, only references with full text available were screened and an additional criteria was used: the document provides a detailed description of the surveillance organisation and

operations. Article meeting all inclusion criteria were registered. Bibliographies of selected publications were reviewed to identify other relevant references.

Data extraction

The selected surveillance systems were assessed regarding 38 variables, allowing the description of the organisation, the functioning, the surveillance context, the health hazards and population under surveillance, the type of collaboration and underlying mechanisms, the barriers and favouring factors to the collaborations in place, the performance and benefits of the systems (See Table 1). Data extraction was conducted using a spreadsheet and then a database was built up by capturing data into an electronic-based multi-choice questionnaire, with pre-defined modalities. In case insufficient data were found in the retrieved documents, additional searches were conducted on the internet to fill up the information gaps.

Data analysis

To identify a potential typology for existing OH surveillance system, a multi-variate analysis was applied on a sub-set of 12 variables (see Table 1). Variables were chosen for their ability to describe important system features and to differentiate systems from each other. For some variables, original modes were grouped in new modes to reduce their number and increase their contingency. Three surveillance systems, with lacking information regarding the selected variables were excluded. A data matrix was computed, included 38 surveillance systems (individuals) and 12 categorical variables. A multiple correspondence analysis (MCA) was then conducted to explore potential associations between the existing surveillance systems and the selected variables (Dohoo et al., 1997). Fisher's exact tests were applied on variables suspected to be highly correlated to others. If the correlation was statistically confirmed, variables were added as supplementary variables. The MCA was conducted with the statistical software Rstudio, using the package FactominR.

Hierarchical cluster analysis (HCA) was then used to differentiate between systems with similar profiles. HCA was conducted on the factorial coordinates of the individuals provided by the previous MCA, using the HCPC function of FactominR (Anonymous 2017a). A distance matrix was computed based on the individual coordinates on the first three main factors (representing 36% of the variance) from the previous MCA and using the Euclidean distance.

RESULTS

The literature search identified a total of 1,635 records. After the screening phase, 31 references were kept and 22 additional references retrieved from bibliographies were added. From these 53 selected documents, 41 surveillance systems were retrieved in line with the set definition (see Table 2).

General organisation and functioning of the existing One Health surveillance systems

The analysis of the 21 first variables underlines some trends in the general organisation and functioning of existing surveillance systems. The coordination of the surveillance systems mainly involved governmental authorities (75.6%). In most of the cases (56.1%), a single institution is leading the coordination. The public health sector leads alone 56.5% of the systems, among which are multi-components systems (84.6%) including three to four data sources. In surveillance systems with multi-institutional coordination, the most common number of co-leading institutions is two (72.2%), and mostly engaged the animal health and

the human health sectors (76.9%). Surveillance objectives are either early detection (36.6%) or trends/occurrence monitoring of health events (43.9%) or both (19.5%). Two systems additionally aim to demonstrate freedom. The primary pursued purposes are diverse: timely response to ensure rapid response (43.9%), improving epidemiological knowledge and health hazards characterisation (19.5%), supporting the design or the efficacy evaluation of risk mitigation interventions (22.0%), eradication or control of a health hazards (7.3%), rapid risk assessment (7.3%).

The hazards under surveillance in the systems retrieved are food-borne diseases (12.2%), antibiotic resistance (24.4%), vector-borne diseases (26.8%) and non food-borne and vector-borne zoonotic diseases (31.7%). When surveillance systems focus on a single hazard (73.2%), West Nile virus (30.0%) and antibiotic resistance (30%) are the most represented. Only two systems (4.9%) surveyed environmental hazards. Number of data sources range from one to four and the most frequent number of surveillance components is three (43.9%). The most frequent combination of data sources (26.8%) is a simultaneous collection in domestic animals, environment, humans and wildlife.

Most of the surveillance systems (70.7%) have been established before 2007, the date from which the concept has been acknowledged by many governmental organisations. The retrieved surveillance systems are implemented at a sub-national (26.8%), national (65.9%) or supra-national level (7.3%). They are usually conducted in high income countries (70.7%). The majority of the OH surveillance systems (82.9%) resulted in *a posteriori* rapprochement of sectoral surveillance components. Very few were established in a collaborative way from the beginning. For some of them, the organisation is not stable and the number of sectoral components vary over time.

Dimensions and degrees for collaborations in the One Health surveillance systems

The analysis of the existing systems led to the identification of several domains at which collaborations across sectors and disciplines may occur (variables 23 to 31 of Table 1): (i) institutional collaborations for the governance of the surveillance system; (ii) collaborations at the different scales of the decision-making process; (iii) collaborations across disciplines coming from biosciences, social sciences and engineering; (iv) collaborations through public-private partnerships; (v) operational collaborations for the implementation of the surveillance activities; (vi) collaborations for the dissemination and the communication of the surveillance results.

At the governance level, collaborations can occur across institutions for the coordination and/or the operations of the surveillance process. In most of the cases, when the coordination is led by a single sectoral institution, collaborations are established with institutions concerned by all the different populations targeted by the surveillance system. When described, the main mechanisms supporting collaborations are the setting up of an inter-agency committee or the existence of official documents describing the role and responsibilities of the actors at stake. For collaborations occurring at the operational level for the implementation of the surveillance activities, several degrees of collaborations were identified at all the different steps of the surveillance process: planning, data collection (including sampling, laboratory testing, data reporting), data management (including data sharing), data analysis/interpretation and results dissemination (see Fig. 1). Data retrieved were insufficient to allow a detailed description of collaborations across decision-making scales and through public-private partnerships. Two documents report the implication of the community in the implementation of the surveillance

system. Reported private partners are mainly veterinarians, physicians, private laboratories, farmers, feed/food operators and pharmaceutical companies.

Table 1. Variables used for the characterisation of the surveillance systems.

Level		Variable
Coordination of the surveillance system	1	Mono or multi-institutional coordination
	2	Number of institutions in charge of the coordination[a]
	3	Type of institutions involved in the coordination
	4	Administrative level in charge of the coordination[a]
	5	Number of sectors involved in the coordination[a]
	6	Type of sectors in charge of the coordination[a]
Geographical area	7	Level of coverage of the surveillance (supra-national, national, subnational)
	8	Territory under surveillance
Date	9	Year of establishment of first collaborative efforts
General organisation	10	Status of the surveillance system (stand alone or part of a programme)
	11	Origin of funds (state, private, external, etc.)
	12	Sustainability of funding
	13	*A priori* or *a posteriori* integration of sectoral surveillance components
Objectives and purposes	14	Objectives of the surveillance system[a]
	15	Purposes of the surveillance system[a]
Hazards under surveillance	16	Number of hazards (mono or multi-hazards)[a]
	17	Type of hazards[a]
	18	Communicability of hazards under surveillance
Compartments under surveillance	19	Type of compartments under surveillance
	20	Number of compartments under surveillance[a]
	21	Data sources in each compartment
	22	Type of data in each compartment
	23	Epidemiological status in each compartment
Terminology	24	Terms which are used in the article to describe the inter-sectoral and inter-disciplinary collaborations
Type of collaborations	25	Type of sectors collaborating along the surveillance process[a]
	26	Mechanisms in place to support institutional collaborations
	27	Decision-making scales involved in surveillance activities
	28	Private actors involved in surveillance activities
	29	Type of collaborative efforts for surveillance activities[a]
	30	Mechanisms in place to support collaborations for surveillance activities
	31	Type of collaborative efforts for the dissemination of surveillance results
	32	Mechanisms in place to support collaborations for dissemination/communication of surveillance results
	33	Type of disciplines involved in the surveillance process
Factors influencing collaborations	34	Favouring factors to collaborations
	35	Barriers to collaborations
Performance of the surveillance system	36	Elements supporting evidence of a good performance of the system
	37	Elements supporting evidence of a bad performance of the system
Benefits	38	Elements supporting evidence of benefits coming from the collaborations

[a]Variables used in the multi-correspondence analysis

Table 2. Characteristics of the clusters grouping the One Health surveillance systems.

Clusters	Characteristics of clusters	Name of the One Health surveillance system	References
Cluster 1	Hazard under surveillance: vector-borne diseases Institutional coordination: Public health sector Number of data sources: 4 Objective: Early detection Purpose: Rapid response	The Surveillance of West Nile Virus in France	Ministry of Health, 2012 (circulaires.legifrance.gouv.fr/pdf/2017/04/cir_42120.pdf)
		The Surveillance of West Nile Virus in Vojvodina (Serbia)	Petric et al., Mol Cell Probe 2017 37:28-36
		The Surveillance of West Nile Virus in Saskatchewan (Canada)	Shuai et al., Int J Health Geogr 2006 5:17; Epp et al., 2008 Transbound Emerg Dis 55:411–416
		The Salmonella Data Bank for Routine Surveillance in Brandenburg (Germany)	Talaska et al.,1994 Bull World Health Organ 72 (1): 69-72
		The West Nile Virus Integrated Surveillance System in Greece	Marka et al., Int. J Environ Res Public Health 2013 10:6534-6610
		The West Nile Virus Integrated Surveillance System in the Emilia-Romagna Region	Angelini et al., Euro. Surveill 2010, 15, 19547; Bellini et al., Euro. Surveill. 19, 20953.
		West Nile Virus Surveillance in Italy	Rizzo et al., Euro Surveill 2012, 17, 20172; Napoli et al., Biomed Res Int 2015:1-8; Rizzo et al., Euro Surveill 2016, 21, 30340
		The Surveillance of West Nile Virus in the United States (ArboNET)	CDC, 2013 (https://www.cdc.gov/westnile/resources/pdfs/wnvguidelines.pdf)
		California Mosquito-Borne Virus Surveillance and Response Plan	Brown E.G., 2012 (http://westnile.ca.gov/downloads.php?download_id=3744&filename=2017%20CA%20Response%20Plan.pdf)
		The Surveillance of West Nile Virus in England and Wales	Morgan et al., Immunol Med Microbiol 2006 48:305–312
		Surveillance of West Nile Virus in the United States in the Military Population	Witt et al., Mil Med 2004 169:421-428

Clusters	Characteristics of clusters	Name of the One Health surveillance system	References
Cluster 2	Hazard under surveillance: zoonotic diseases Objective: early detection Institutional coordination: public health sector	Surveillance of zoonotic diseases in the Russian Federation	McNamara et al., Biosecur Bioterror 2013 11(3):185-195
		The Electronic Integrated Disease surveillance System (EIDS)	Wahl et al., Onderstepoort J Vet Res 2012 79(2), Art. #455
		The surveillance of Rabies in Ethiopia	Coetzer et al., Antiviral Res 2016 135 74:80
		The inter-sectoral surveillance of zoonotic diseases in Mongolia	Batsukh et al., Curr Top Microbiol Immunol 2012 82(53)
		The surveillance of schistosomiasis in Guangxi (China)	Sleigh et al., Bull World Health Organ 1998 76 (4):361-372; Sleigh et al., Bull World Health Organ, 1998, 76 (5):497-508
		Global Early Warning and Response System (GLEWS)	OIE, 2006 (https://www.oie.int/doc/ged/D11304.PDF)
		The Human Animal Infections and Risk Surveillance (HAIRS)	HAIRS, 2013 (http://www.hse.gov.uk/aboutus/meetings/committees/acdp/140213/acdp_100_p4c.pdf); Morgan et al., Epidemiol. Infect. 137, 1521.
		The AFHSC - Division of GEIS operations predictive surveillance programme	Witt et al., BMC Public Health 2011 11(Suppl 2):S10
Cluster 3	Institutional coordination: public health and animal health sectors independently Operational collaborations: cross-sectoral data sharing during the surveillance process	The surveillance of *Campylobacter* in Switzerland	Martins et al., Epidemiol. Infect 2017 145(6):1148-1158
		National Observatory of the Epidemiology of Bacterial Resistance to Antibiotics (ONERBA)	ONERBA, 2016 (http://onerba.org/onerba-2015/)
		The Swedish Antimicrobial Resistance Monitoring programme (STRAMA/SVARM)	SWEDRES, 2016 (http://www.sva.se/globalassets/redesign2011/pdf/om_sva/publikationer/swedres_svarm2015.pdf)
		The Dutch Integrated Antimicrobial Resistance Monitoring Programme (NethMap/MARAN)	SWAB, 2016 (www.swab.nl/swab/cms3.nsf/uploads/.../NethmapMaran2015%20_webversie.pdf); Queenam et al., Int J Antimicrob Agents 2016 2016 48(4):422-427
		The surveillance of rabies in Bohol (Philippines)	Lapiz et al., PLoS Negl Trop Dis 2012 6(12): e1891
		The Animal Health Information Network in Canada	Roth, D., 2011 (http://www.ncceh.ca/sites/default/files/Surveillance_Emerging_Infectious_Diseases_Dec_2011_0.pdf)

Clusters	Characteristics of clusters	Name of the One Health surveillance system	References
		Surveillance of Rift Valley Fever in West Africa	EMPRES, 2000 (www.fao.org/3/a-x9550e.pdf
		Influenza surveillance systems in Taiwan	King et al., ICS 1219 (2001) 107–118
		The surveillance of zoonotic diseases in NSW	Adamson et al., NSW Public Health Bull 2011 22:5-6
		The surveillance of rabies in Tamil Nadu (India)	Abbas et al., Int Health 2011 3(4):231-239
Cluster 4	Hazard under surveillance (antibiotic resistance and food-borne diseases); Objective (following trends and occurrence); Institutional collaborations (Animal health, Public health, Food safety)	The surveillance of zoonotic diseases in European Union	Ammon et al., Int J Food Microbiol 2010 139:43-47
		Canadian Integrated Programme for Antimicrobial Resistance Surveillance (CIPARS)	Grant et al., 2014 (http://www.deslibris.ca/ID/244350); CIPÄRS, 2015 (https://www.canada.ca/en/public-health/services/surveillance/canadian-integrated-program-antimicrobial-resistance-surveillance-cipars/cipars-2014-annual-report-summary.html)
		Antibiotic resistance programme in the European Union	JIACRA, 2015 (http://www.ema.europa.eu/docs/en_GB/document_library/Report/2015/01/WC500181485.pdf)
		The integrated salmonella surveillance programme in Canada	Vrbova et al., Epidemiol Infect 2016144:2165–2175
		National antimicrobial resistance monitoring system in the United States (NARMS)	NARMS, 2016 (https://www.fda.gov/downloads/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/UCM581468.pdf)
		The surveillance of *Salmonella* in France	Danan et al., Epidemiol Infect 2011 139:736–741; David et al., Revue Med Vet 2011 162(10):489-500
		The Danish integrated antimicrobial resistance monitoring programme (DANMAP)	Wielinga et al., Food Control 2014 40:185-192; DANMAP, 2016 (https://www.danmap.org/~/media/Projekt%20sites/Danmap/DANMAP%20reports/DANMAP%20%202015/DANMAP%202015.ashx)
		The surveillance of *Trichinella* in Canada	Polly et al., 2000 Vet Parasitol 93:351–363
		The Colombian integrated programme for antimicrobial resistance surveillance (COIPARS)	Donado-Godoy et al., Zoonoses Public Health 2015 62 (suppl. 1):58-69

	-	Increasing degrees of collaboration				+
Planning	Done separately in each sector	Done by a single sector for all components	Cross-sectoral consultation but done separately	Done by a multi-sectoral organisation		
Data collection	Done separately in each sector	Done by a single sector for all components	Harmonisation across sectors	Joint sectoral activities	Done by a multi-sectoral organisation	
Data sharing	No data exchange	Notification of unusual events only	Continuous data exchange			
Analysis/ Interpretation	Done separately in each sector	Done separately and then compared by a single sector	Jointly done by a single sector for all components	Done separately and then compared by a multi-sectoral organisation	Jointly done by a multi-sectoral organisation	
Dissemination	Done separately for each sector	Joint dissemination in separate sectoral activities	Joint dissemination by a single sector	Joint dissemination by a multi-sectoral organisation		

Fig. 1 Possible degrees of collaborations at the different steps of the surveillance process.

Factors influencing the collaborations

Factors that have influenced positively the implementation and the functioning of a collaborative surveillance system are mentioned for 21 systems (51.2%). Factors related to the existence of an appropriate framework to ease collaborations across sectors are the most numerous (57.1%): existence of an overarching OH programme in which the surveillance system is embedded, appropriate legal framework or institutional organisation, existing institutional culture for inter-sectoral collaborations, clear definition of roles and duties of all the actors at stake, existence of inter-sectoral collaborations mechanisms at a supra-level providing a frame for collaborations at an infra-level, supervision by the same authority of the sectors in charge of surveillance components. For some systems, collaborations are not supported by an official framework but by preferential relationships existing between individuals working in different sectors and disciplines. Other favouring factors are related to mechanisms ensuring the commitment of the stakeholders, at the political and operational level: efficient and appropriate communication and consultation channels, ability of the system to meet the objectives of the different stakeholders. Epidemiological factors are also mentioned to explain the motivation to establish collaborations for surveillance activities, such as the scientific evidence of the efficiency of using animal sentinels or vectors surveillance components to protect human health, or the necessary recognition of the interconnectivity between compartments in the conception of an efficient surveillance system. Technical factors may also be involved in the success of a collaborative surveillance system: availability of a joint database, easiness of data exchanges thanks to compatible sectoral information systems, existence of a fully functional national reference laboratory.

Barriers hampering operations of the collaborative surveillance systems have been specified for 14 systems (34.1%). They are essentially technical barriers (78.6%): lack of standardisation and harmonisation for the data collection, incompleteness of the data, insufficient data sharing across sectors including unreliable cross-sectoral alert system, incomplete multi-compartment

data analysis and interpretation. In some cases, the collaboration might have not reached a sufficient level because of the absence of engagement of the private sector or an insufficient integration with some sectoral compartments still conducted separately. As a result, the systems cannot meet their objective, such as the detection of health events in animals to prevent human cases or the attribution of sources for human cases of food-borne diseases. In addition, legal constraints are also mentioned (42.9%): the property and confidentiality of data, ethical issues, inadequate legal and operational framework to clearly define roles and mandates of the different actors involved and to support collaborations at the ground level. Inappropriate amount and allocation of resources are also hampering collaborative approach. On one hand, budget is vertically allocated and should benefit the sector to which it was allocated; on the other hand, there is no specific resources available for inter-sectoral activities. Additionally, resources are scarce, especially for surveillance activities and actors may have to compete to access them, which is further reinforcing the lack of collaborations.

Typology of existing surveillance systems

The MCA was finally conducted with 10 variables among the 12 initially selected. Two variables were considered as supplementary variables (namely number of sectors involved in the coordination and number of compartments under surveillance) because of their high correlation with other variables (respectively type of sector in charge of the coordination and type of compartments under surveillance).

Variables representing most the inter-individual variance are: “hazard under surveillance”, “purpose”, “type of sectors in charge of the coordination” and “type of sectors collaborating along the surveillance process”. The two extreme modalities on the first axis are “antibiotic resistance” and “vector-borne diseases” for the variable “hazard under surveillance”, “knowledge” and “rapid response” for the variable “purpose”, “mono-sectoral coordination (led by the public health sector)” and “multi-sectoral coordination (involving the animal health, public health and food safety sectors)” for the variable “type of sector in charge of the coordination”.

The HCA applied on MCA results shows clearly that surveillance systems are clustering in four distinct groups regarding the three first axis. Table 2 lists the surveillance systems included in each of the clusters as well as the variables and respective modalities characterising those clusters. The first one includes 90% of systems monitoring vector-borne diseases. They usually aim at detecting early cases to trigger a rapid response (90%).The coordination is mainly mono-institutional and led by the public sector (70%), even if collaborations are developed with other sectors concerned by the populations under surveillance. At the operational level, collaborations concern both data sharing and data analysis/interpretation. A second cluster includes only systems monitoring zoonotic diseases, mainly with the objective to early detect positive cases (87.5%). 75% of them are coordinated by the public health sector, undertaking collaborations with other sectors specialised in animal health, food safety and environmental sciences. All the systems aiming at rapid risk assessment are part of this cluster. At the operational level, they all show at least data sharing across sectors during the surveillance process. The third cluster consists of systems that include sectoral surveillance components supervised independently by the animal and the human health sectors. While hazards and surveillance purposes are diverse, most of them (90%) demonstrate collaborations in terms of cross-sectoral data sharing during surveillance campaigns. Finally, a last cluster includes systems monitoring antibiotic resistance and food-borne diseases. Those systems aim at following trends and occurrence to improve the epidemiological knowledge and/or provide appropriate data for research projects. The coordination pattern varies a lot within this cluster,

but collaborations are always in place during the surveillance process across institutions in charge of animal health, public health and food safety. Operational collaborative efforts focus on joint or compared analysis of data coming from the different sources (100% of the systems).

DISCUSSION

The systematic literature review retrieved 41 existing surveillance systems, in which collaborations across sectors and disciplines may occur at the different steps of the surveillance process to a various degree. These systems are mainly characterised by the hazard under surveillance, the surveillance purpose, the type of sector leading the coordination and the type of sectors involved in the surveillance activities.

However, these results should be interpreted in light of a number of biases related to the retrieval methodology of the documents describing the surveillance systems. Many surveillance systems, and especially those established for official purpose do not necessarily lead to publications and so might be not included in the study. During the review, some documents referring to the study's definition of a OH surveillance system were retrieved first but then excluded from the analysis as they were not providing enough information. Moreover, organisation of some systems may have evolved since the publication and data used for the analysis might be outdated. Finally, some documents have been published by authors who are not involved in the coordination or implementation of the surveillance system (review article about surveillance systems) and as a result, may not be fully aware of mechanisms underlining the surveillance organisation; on the contrary, some authors might be subjective when describing the satisfactory performance as well as the added-value of the system they supervise, especially in absence of provision of clear evidence.

The HCA applied on the MCA showed four distinct clusters of surveillance systems with clear pattern in terms of collaborations depending on the surveillance objective and context. In surveillance systems aiming at early detecting positive cases to trigger rapid response or to eradicate/control health hazards (clusters 1 and 2), they all demonstrate collaborative efforts in terms of data sharing during the surveillance campaign. For surveillance systems specifically monitoring vector-borne diseases (cluster 1) which collect data coming from three to four data sources, additional collaborations across sectoral institutions happen at the data analysis step. Cross-sectoral data sharing can be considered as the minimal level of collaborations required for surveillance systems aiming at early detecting cases, which makes sense to ensure a timely response in populations at risk. When the system includes many components and data sources, further collaborations in terms of data analysis and interpretation seem to be required. This is illustrated with the examples of rabies and West Nile virus. Surveillance systems dedicated to rabies usually include a single passive surveillance component in humans and domestic animals (usually dogs); collaborations are reduced to cross-sectoral alerts of positive cases to allow each sector to take appropriate actions to reduce the risk within the scope of their regulatory and jurisdictional authorities. On the contrary, West Nile virus surveillance is usually more complex, including active and passive components targeting various populations (humans, domestic animals, wildlife and vectors) as well as collecting different types of data (confirmed laboratory, ecological and epidemiological data, suspected cases); most of them demonstrate collaborations across sectors at the data analysis and interpretation step in order to develop appropriate targeted mitigation measures. In systems focusing on monitoring trends and occurrence of health hazards, collaborations mainly take place at the data analysis and interpretation stage while data sharing during the collection stage is rare (cluster 4). Data sharing does not appear as a collaborative need in these systems which mainly aim at improving

epidemiological knowledge and at supporting development and evaluation of interventions to mitigate the risk. Collaborations is valuable at the analysis and interpretation step where knowledge and competencies from the different sectors and disciplines are required to make the best use of the data. Hazards targeted by these systems are food-borne pathogens or antimicrobial resistance, for which the detection of positive cases in one population does not necessary call for immediate actions in other populations to reduce the risk. However, a comprehensive knowledge of the route of transmission and a clear determination of sources attribution are necessary to develop appropriate interventions in the related sectors of concern and only a multi-disciplinary approach is able to reach this objective.

In the last decade, the OH concept has been endorsed and largely promoted at the global and local level. Despite the persistence of silo thinking, many initiatives have emerged. In terms of surveillance, this study suggests that efforts mainly focus on the prevention of zoonotic diseases (including vector-borne diseases and food-borne diseases), and more recently of antimicrobial resistance. The review has retrieved only two articles describing surveillance initiatives bringing together health and environmental specialists in an effort to address health risks related to environmental contaminants. Nevertheless, environmental contaminants, such as heavy metals, dioxins, PCB, myco- and phycotoxins, are quintessential OH issues. Animals and humans share the same environment and the same sources of food and water; they are potentially exposed to the same chemicals. Additionally, human can be contaminated through the ingestion of contaminated animal products, which are an important part of the human diet (Buttke et al, 2011). Environmental contamination is so calling for highly interdisciplinary approach to respond appropriately to the related health risk

In many studies, the OH concept is assimilated to an integrated approach. The definition of integration is the act of combining or adding parts to make a unified whole (Anonymous 2017b). Applying this definition to surveillance implies that data from different sources are jointly collected and/or combined. The concept of collaboration, a fundamental principle of the OH concept (Zinsstag et al. 2011), is therefore not intrinsic to an integrated surveillance system. Thus, a surveillance system can be described as integrated but not considered as OH if it collects and combines data coming from several domains without demonstrating collaborations across sectors and disciplines. For instance, a sectoral institution can collect, analyse and interpret data collected in compartments that are not falling in their competencies field, without seeking for collaborations with institutions in charge of those compartments (McNamara et al., 2013). Using interchangeably one term for another is confusing and does not support effective operationalisation of the OH concept in the field of surveillance.

The findings of this review reinforce the hypothesis that a conceptual framework is missing to define exactly the notion of OH surveillance and this hampers the operationalisation of collaborative efforts for effective and sustainable surveillance systems.

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SYNDROME TIME SERIES SELECTION BASED ON ABERRATION DETECTION PERFORMANCE

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SUMMARY

The aim of this study was to estimate the potential value of different time series (TS) for implementing a syndromic surveillance system. Simulations were used to produce outbreaks of different size and shape in order to estimate the capacity of each time series to detect them with high sensitivity, specificity and timeliness. Two temporal aberration detection algorithms were compared: Holt–Winters generalized exponential smoothing (HW) and Exponential Weighted Moving Average (EWMA). The results indicated that a specific aberration detection algorithm should be used for each TS. In addition, some TS had poor detection performance and detected only large outbreaks making them of limited use for early detection. They could however be transformed to improve their detection performance, or used for other surveillance purposes.

INTRODUCTION

Syndromic surveillance (SyS) is a relatively new method of health surveillance that was developed to enhance traditional passive surveillance systems. SyS is real-time or near real-time surveillance based on pre-diagnostic often unspecific routinely collected data which is available prior to laboratory confirmation of an epidemic. Currently, SyS systems in animal health monitor several to many individual syndrome TS. However, the analyses is usually univariate, meaning that each individual syndrome TS is monitored independent of the other TS in the system.

Building syndromic surveillance systems is not a straight forward process. The question of identifying and subsequently analysing the appropriate syndromes to monitor is very challenging and has been recently identified as one of the research priority areas in syndromic surveillance (Hopkins et al., 2017). Selecting appropriate syndromes to monitor is especially challenging when the data allow numerous syndromes classifications or definitions (Hopkins et al., 2017), and when the objectives of surveillance are unspecific. Vial and Berezowski have suggested that the selection of syndromes should be made in accordance with the objectives of the surveillance system (Vial & Berezowski, 2015). However when the objectives are broad (e.g., to detect any new disease), they are of little help for selecting the most appropriate syndromes.

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The ability to detect an epidemic with specific characteristics in a syndrome TS (referred to here as the detection performance of a syndrome TS) is likely to be affected by the characteristics of the event detection algorithm, the TS, and the characteristics of the epidemic. Assessing the nature of the change that could potentially be detected in a syndrome TS could be used as a criterion for syndrome selection. The syndrome TS used in surveillance are highly variable in terms of the mean number of cases per unit of time, the variance in the number of cases per unit of time, day of the week effect, seasonality and long term trend. Any syndrome used in combination with any aberration detection algorithm should be able to detect a large epidemic that has an extreme increase in the number of reported syndrome cases. Detecting extreme epidemics however is of little interest for surveillance of infectious disease that can rapidly spread in a population, as the disease will be wide spread by the time it is detected as a large epidemic. In this case the aim of surveillance is the early detection of any small change occurring in the syndrome TS that could be the early stage of an epidemic. Evaluating the minimum size of an outbreak that can be detected in a specific syndrome TS could be used for comparing the performance of different syndromes and for providing some guidance for syndrome selection.

The minimum size of the epidemic that can be detected in a syndrome TS can only be estimated through data simulation. Different outbreak simulation methods are available : a) the number of extra epidemic cases added to the baseline TS is based on real epidemic data, b) the number of extra epidemic cases added to the baseline TS are multiples of the standard deviation of the baseline TS or c) the number of extra cases are defined in vectors containing a fixed number of extra cases (Lotze et al., 2007). The first method is often not possible because data describing the shape and size of an epidemic in a specific syndrome TS rarely exists. In addition, the method is irrelevant when the surveillance objective is to detect an unknown disease or many different diseases. The second approach is appropriate for comparing different algorithms applied to the same TS, but it is not possible to use this method to compare the detection performance of the same algorithm in different syndrome TS. To date, the last approach has been rarely implemented, but it is the only one that could potentially be used to compare the detection performances of different syndrome TS.

The objective of this study was to compare the detection performance of 16 syndromes extracted from 3 different data sources being considered as candidates for a national Swiss SyS system. Simulated epidemics of different size and shape were used to estimate the epidemic detection performance of each syndrome TS. Performance metrics used were sensitivity, specificity and timeliness. Two univariate temporal aberration detection algorithms were also compared. The minimum size of an epidemic that could be detected by each syndrome was then computed and used to draw conclusions about the potential benefits and drawback of using each syndrome in a SyS system.

MATERIALS AND METHODS

Data sources and associated TS

Sixteen syndrome TS were extracted from three databases containing national level data from the Swiss cattle population.

- The *Swiss animal movement database* (AMD) contains data on cattle mortalities reported by farmers to the national Swiss system for the identification and registration of cattle. The AMD has already been shown to have potential value for syndromic surveillance because

of its relatively high quality in terms of population representativeness and case reporting timeliness (Struchen et al., 2015). Four TS were created; one containing stillbirths (*AMD_stillbirth*) and 3 containing on-farm deaths defined according to the age at death: up to 6 month old (*AMD_mortality_calves*), 6 month – 2 years (*AMD_mortality_young*), and more than 2 years *(AMD_mortality_adults*). Data were available from January 2009 to December 2016.

- The *Association of Swiss Cattle Breeders (ASR) (http://asr-ch.ch/en/asr/)* is the private umbrella organisation of the Swiss cattle breeding organisations. Since 2013, ASR has developed and implemented a homogenized database collecting farmer's diagnostic findings. Three TS were first created based on the type of animal diseased: abortion (*ASR_abortion*), diseased calves up to 6 month old (*ASR_calves*), or diseased adults (*ASR_adults*). Then, *ASR_calves* and *ASR_adults* were also respectively split into 3 TS based on the most frequent clinical conditions found in the database: gastrointestinal symptoms (i.e., *ASR_GI_calves* and *ASR_GI_adults*), respiratory symptoms (i.e., *ASR_RESPI_calves* and *ASR_RESPI_adults*), and cattle showing a pathology defined as "other" in the ASR classification schema (i.e., *ASR_OTHER_calves* and *ASR_OTHER_adults*). The category "other" encompasses various unspecific symptoms such as fever, anorexia, changing behaviour or reduction of production. ASR data were available from January 2014 to December 2016.

- The *Swiss laboratory information system* (ALIS) contains data from laboratory tests performed by the 25 recognized laboratories involved in the diagnosis of epizootics in Switzerland on behalf of the Swiss Veterinary Services. All the laboratory tests performed for the 69 notifiable epizootics of interest in Switzerland are collected in the ALIS database and the population coverage of these data are thus high. All laboratory tests performed for mandatory reasons without any clinical suspicion of disease were excluded (e.g., mandatory surveillance program, importation, vaccination, research activities). A first TS was created based on the samples sent because of abortion *(ALIS_abortion)*. Two additional time-series were then created based on the samples sent because of clinical suspicions of two diseases of interest for Switzerland, Bovine Virus Diarrhoea (*ALIS_BVD*) and Infectious Bovine Rhinotracheitis (*ALIS_IBR*). Data were available from November 2013 to December 2016.

Time series analysis and preprocessing

Weekly TS were generated and their temporal patterns (e.g., trend, season) and mean weekly number of cases were computed. Sporadic but obvious outlier values were identified by visual examination in the datasets. These abnormal values affected only 1 week in *ALIS_abortion*, and 6 weeks in *ALIS_IBR*. In order to work with outbreak-free TS, these aberrations were removed and replaced by the weekly average of the 10 previous weeks.

Aberration detection algorithms

Two different univariate aberration detection algorithms were compared: Holt–Winters generalized exponential smoothing (HW) (Chatfield & Yar, 1988; Gardner, 1985) and Exponential Weighted Moving Average (EWMA) (Hunter, 1986). For both algorithms 10 different detection limits were tested and, to avoid contamination of the baseline with gradually increasing outbreaks, a guard-band length of two weeks was used between the baseline and the current values being evaluated.

HW can be applied to raw data containing trend and seasonality. The optimal HW parameters were determined through minimisation of the squared prediction error. EWMA should only be applied to stationary and normally distributed data. A one week differencing was implemented to remove the most important temporal effects present in the raw data and the differenced residuals (the residual at each time point *t* being the difference between the observed value at *t* and *t-1*) were saved as a new TS. EWMA was then applied to this new TS using a smoothing coefficient of 0.2.

Data simulation

Three hundred outbreak-free baselines for each TS were simulated. The mean fitted value for each week of the year 2016 previously obtained with the best HW model was used as the mean of a Poisson distribution. Random samples were made from this distribution to simulate the outbreak-free baselines.

Twenty five different outbreak signals were simulated based on different outbreak shapes and magnitude. Five outbreak shapes representing different temporal progressions of an epidemic within a population were tested, including: single spike, moving average, linear, exponential and log normal. Five different outbreak magnitudes were tested: 25, 50, 150, 300, and 500 cases, corresponding respectively to very small, small, medium, large and very large epidemics. The cases are the maximum number of extra cases added to the outbreak-free baseline during an epidemic time period. Outbreak length was assumed to be constant and equalled to 12 weeks. Three hundred outbreaks of each type were simulated and randomly inserted within the 300 simulated baselines. Only one outbreak was inserted per simulated baseline to avoid outbreak overlap.

Detection performance estimation

Firstly, sensitivity was calculated based on the number of outbreaks detected out of all inserted outbreaks and denoted this *Se*. The specificity (*Sp*), the positive predictive value (*PPV*) and the negative predictive value (*NPV*) were also calculated. A receiver-operating characteristic (ROC) curve was generated and, assuming equal costs of false negative and false positive alarms, the optimal alarm threshold was graphically defined where *Se* and *Sp* are maximum. The timeliness of the first alarm raised during an epidemic time period was computed. Detection timeliness was expressed in terms of time lag between the start of the epidemic and the first alarm. A value of 1 would thus mean that the first alarm was raised during the second week of the epidemic. Single spikes were excluded from the computation of the detection timeliness as they always leads to a detection on the first, and unique, week of the epidemic.

RESULTS

Time series analysis

All 16 TS under study had significant seasonal effects and 7 of them also presented a long term trend. The TS peaked however at different seasons. The main difference between these TS were the length of historical data available, which ranged from a bit less than 3 years for ASR to more than 7 years for AMD. The average number of reports per week varied from 2.6 cases per week for *ASR_OTHER_calves* to 892 cases per week for *AMD_mortality_calves*.

Algorithm comparison

As expected, both aberration detection algorithms performed better when detecting large epidemics (i.e., higher sensitivity, specificity and detection timeliness). Regarding the outbreak shapes, flat increases were detected with higher sensitivity, specificity and timeliness than log normal and linear increases. Single spikes and exponential increases had the worst performance and were the outbreaks shapes most difficult to detect for both algorithms.

The performance of the two algorithms differed depending on the TS considered, but no difference was observed according to the outbreak shape. Both algorithms showed equivalent sensitivity and specificity for 3 TS: *ASR_abortion*, *ASR_OTHER_calves*, and *ALIS_BVD*, (see Fig. 1). However, HW outperformed EWMA for 11 TS: *AMD_stillbirth*, *AMD_mortality_calves*, *AMD_mortality_adults*, *ASR_OTHER_adults, ALIS_abortion, ASR_GI_calves, ASR_calves, ASR_RESPI_calves, ASR_RESPI_adults, ASR_GI_adults*, and *ASR_adults*. EMWA outperformed HW for only 2 TS: *AMD_mortality_young*, and *ALIS_IBR*. HW showed an equivalent or a better balance between the detection timeliness and the average number of false positive alarms than EWMA in most of cases. However, the EWMA algorithm had better timeliness for *ALIS_BVD, ALIS_IBR* and *AMD_mortality_young*.

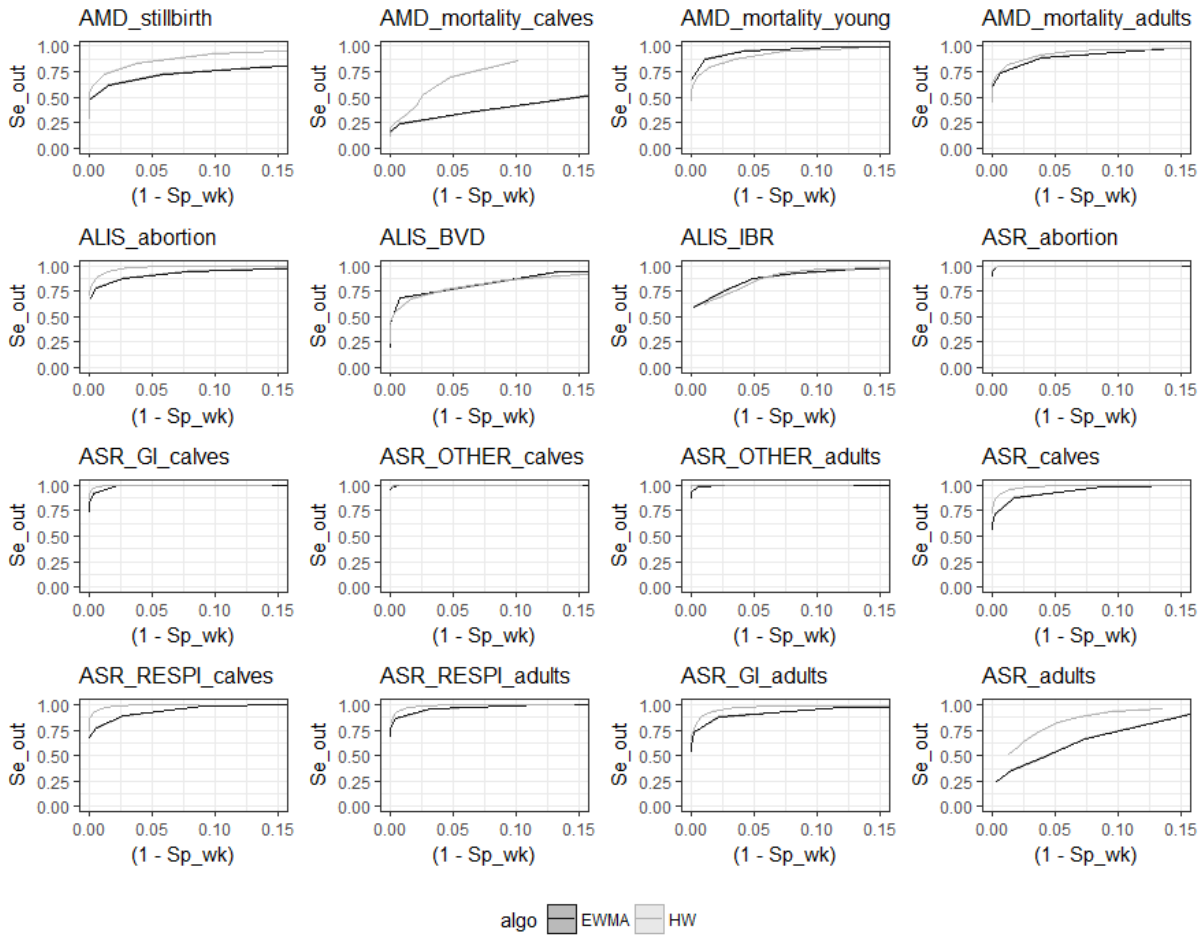

Fig. 1 Receiver-operating characteristic (ROC) curve for the 16 syndromes and the 2 aberration detection algorithms (all outbreak sizes and shapes merged).

AMD_stillbirth, AMD_mortality_calves, AMD_mortality_adults, ASR_OTHER_adults, ALIS_abortion, ASR_GI_calves, ASR_calves, ASR_RESPI_calves, ASR_RESPI_adults, ASR_GI_adults, and *ASR_adults* always obtained better detection performance with HW, and this algorithm was thus considered the optimal algorithm for these TS for the rest of the analyses. *AMD_mortality_young* and *ALIS_IBR* always had better detection performances with EMWA and this algorithm was thus defined as the optimal one for these two TS. The overall sensitivity and specificity of *ALIS_BVD* were equivalent between HW and EWMA but the detection timeliness was slightly better with EWMA. EWMA was thus chosen as the most appropriate aberration detection algorithm for *ALIS_BVD*.

Time series comparison

The optimal cut-point for the optimal algorithm previously selected for each TS was graphically estimated and the detection performances obtained at the optimal alarm threshold were summarized in Tables 1 and 2.

Table 1. Global Detection performances obtained with the optimal algorithm at the optimal alarm threshold. TS are ordered according to their weekly average number of notification from the largest (top row) to the smallest (bottom row). FP/yr = mean number of false positive alarms per year.

Time series	Optimal algorithm	Optimal alarm threshold	Se	Sp	PPV	NPV	FP/yr	Timeliness
AMD_mortality_calves	HW	0.05	85.9	89.8	44.9	84.8	4.0	2.0
ASR_adults	HW	0.5	93.4	90.4	56.5	88.3	3.8	2.1
AMD_Stillbirth	HW	0.5	92.4	90.2	53.1	87.1	3.9	1.8
ALIS_BVD	EWMA	0.5	93.3	86.6	46.6	87.2	5.3	1.9
AMD_mortality_adults	HW	0.75	94.4	93.7	65.4	88.1	2.5	1.6
AMD_mortality_young	EWMA	0.75	95.5	95.7	77.4	90.6	1.7	1.6
ASR_GI_adults	HW	1.5	97.1	96.5	77.6	88.7	1.4	1.4
ALIS_IBR	EWMA	0.75	92.4	91.6	62.2	89.6	3.3	1.4
ALIS_abortion	HW	1.5	97.6	96.9	81.3	89.8	1.2	1,3
ASR_calves	HW	2	96.2	98.0	85.9	88.0	0.6	1.3
ASR_RESPI_adults	HW	2	97.4	98.5	87.3	89.0	0.6	1.0
ASR_RESPI_calves	HW	1	98.4	98.0	88.7	91.7	0.3	1.1
ASR_GI_calves	HW	2.25	98.9	99.0	93.3	89.7	0.4	1.2
ASR_Abortion	HW	3	99.4	99.4	95.7	89.3	0.2	1.2
ASR_OTHER_adults	HW	3	99.9	99.8	98.3	90.1	0.1	1.0
ASR_OTHER_calves	HW	3.5	99.5	99.7	97.4	89.7	0.1	0.9

The optimal alarm thresholds always lead to overall sensitivity and specificity above 85%. The overall average detection timeliness demonstrated that, when an outbreak was detected, the first alarm was raised on average on the second or third week of the epidemics. Detection performance varied however according to the magnitude of the epidemic. Only half of the TS were able to detect very small epidemics (i.e., magnitude 25) with a sensitivity above 85%, but all TS except *AMD_mortality_calves* detected more than 85% of the small epidemics (i.e., magnitude 50).

NPV ranged from 84 to 95% for all TS. However, there were significant variations in the PPV. The 4 TS with the smallest average weekly count had a PPV above 93% (i.e., *ASR_abortion, ASR_OTHER_adults, ASR_GI_calves, ASR_OTHER_calves*). All the other TS had lower PPV, which was even below 50% for *AMD_mortality_calves* and *ALIS_BVD*. These low PPV resulted in 4 to 5.3 false alarms per year.

Table 2. Detection performance obtained with the optimal algorithm at the optimal alarm threshold.

Time series	Very small epidemics (25)		Small epidemics (50)		Medium epidemics (150)		Large epidemics (300)	
	Se	Sp	Se	Sp	Se	Sp	Se	Sp
AMD_mortality_calves	60.6	89.1	71.6	89.3	97.1	89.9	100	90.2
ASR_adults	78.8	86.7	88.1	87.6	100	90.0	100	92.7
AMD_Stillbirth	75.1	88.1	87.0	88.6	100	89.7	100	91.2
ALIS_BVD	79.3	84.0	87.3	84.8	100	86.5	100	88.0
AMD_mortality_adults	78.3	92.0	93.8	92.4	100	93.5	100	94.9
AMD_mortality_young	80.2	94.4	97.4	94.8	100	95.7	100	96.6
ASR_GI_adults	87.0	94.1	98.0	95.2	100	97.6	100	97.7
ALIS_IBR	71.7	89.7	90.4	90.3	100	91.7	100	92.9
ALIS_abortion	88.0	94.0	99.0	96.0	100	97.9	100	97.9
ASR_calves	83.1	96.9	98.0	98.2	100	99.2	100	99.1
ASR_RESPI_adults	87.8	96.8	99.4	98.2	100	99.1	100	99.1
ASR_RESPI_calves	91.9	95.5	100	97.9	100	98.8	100	98.8
ASR_GI_calves	99.9	92.8	100	94.9	100	95.7	100	95.9
ASR_Abortion	97.0	99.3	100	99.4	100	99.4	100	99.4
ASR_OTHER_adults	99.2	99.7	100	99.7	100	99.7	100	99.7
ASR_OTHER_calves	97.4	99.6	100	99.6	100	99.6	100	99.7

DISCUSSION

The results found here demonstrate that no single algorithm should be expected to perform optimally across all syndrome time-series. HW outperformed EWMA in most of the TS (i.e., 13 out of 16 TS) which confirmed the conclusions of (Burkom et al., 2007; Dórea et al., 2013). The better detection performances of EWMA compared to HW with *AMD_mortality_young, ALIS_IBR*, and *ALIS_BVD* might be explained by the high mean weekly count of these 3 TS together with the wide prediction intervals obtained with HW.

It was expected that there would be differences in detection performance for different epidemic shapes between the two algorithms. EWMA is especially adapted for detecting small but repeated differences between observed and expected values as found in flat or linear epidemics (Mandl et al., 2004)(Hunter, 1986)(Dórea et al., 2013). HW, on the other hand, is reported to be more effective for detecting large epidemics with a sudden increase in cases as in peaks or exponential outbreak (Unkel et al., 2012)(Dórea et al., 2013). These differences were not evident in the current study. The varied characteristics of the time-series may have been main factor that influenced the performance of the aberration detection algorithms in this study.

This study results highlighted the difference between the overall detection performance (i.e., all epidemic magnitudes merged) and the specific detection performance obtained for different epidemic magnitudes. The lower detection performance obtained for very small and small epidemics compared to the other epidemic magnitudes was not surprising as small increases can easily remain unnoticed in the background noise of a TS, especially when the TS contains high count data. Other studies have assessed TS detection performances using different epidemic magnitudes but these studies only reported overall detection performance (see for example (Choi et al., 2010; Dórea et al., 2013)). Assessing the detection performance of a TS for different epidemic magnitudes separately appears however essential to assess the real value of an alarm, or a non-alarm, occurring in a TS. To the authors' knowledge, this is the first time that the difference in terms of detection performance between epidemics of different magnitude has been highlighted and quantified.

The results found here question the benefit of using TS with poor detection performance for SyS. In this study, even small and very small epidemics had a substantial number of accumulated epidemics cases. Over the 12 week epidemic period, very small epidemics had an average of 201 (i.e., range from 103 to 300 depending on the epidemic shape) epidemic cases and small epidemics had an average of 386 (i.e., range from 172 to 600 depending on the epidemic shape) epidemic cases. Not able to detect such large numbers of excess cases makes these TS less suitable for early detection of epidemics. If the available data allow, these TS should be modified in order to improve their detection performance. For example, in this study, *AMD_mortality_calves* contains all the calves dead before 6 months of age. If required this syndrome TS could be split in at least 2 TS, one for neonatal calves younger than 1 month and one for calves 1 month to 6 months of age. This transformation would reduce the number of reports per week which could make the TS more sensitive to small changes. It is however not always possible to split all TS, as for example with *AMD_stillbirth*. When it is not possible to split or aggregate the TS, it may be possible to use other methods. When geographical information are available, hierarchical TS approaches (Alba et al., 2015) or other spatiotemporal methods (see for example (Alkhamis et al., 2012; Hyder et al., 2011)) could be used to increase the detection performances. When it is not possible to transform a TS or use other approaches to improve detection, the usefulness of the TS for early epidemic detection remains in question. However, the benefits of SyS are reported to be numerous and go beyond early epidemic detection (Dorea & Vial, 2016). Even if a TS is not suitable for early event detection it may have other uses such as defining the normal behaviour of animal populations in the absence of a specific epidemic or providing additional quantitative information for decision making that may support other surveillance programs.

The results of this study suggest that surveillance system designers should carefully assess each candidate syndrome TS before including it in their early epidemic surveillance system. The assessment should include fitting an optimal event detection algorithm to the TS and then evaluating the detection performance of the TS-algorithm pair on a variety of epidemic types. Only those TS which have acceptable performance for epidemics types that are similar to epidemics of the disease under surveillance should be included in the SyS system.

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VIRAL DISEASES

TOWARDS RISK-BASED CONTROL OF BOVINE RESPIRATORY SYNCYTIAL VIRUS IN NORWAY BY ESTIMATES OF HERD-LEVEL PROBABILITY OF FREEDOM

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SUMMARY

A key strategy in the Norwegian BRSV control program is to identify antibody negative herds, and protect them from introduction of virus. Because infection is endemic, the rate of reintroduction can be high. The aim of this study was to estimate the herd-level probability of freedom from BRSV antibodies over time. Input variables were test sensitivity, the probability of introduction through animal purchase and probability of local transmission. Bulk tank milk was sampled one to three times from herds located in two counties in western Norway throughout the study period (2013-2016). Probability of freedom (*PostPFree*) was generally high for negative herds immediately after testing, but declined with time, and was greatly affected by purchase of livestock. Comparing the *PostPFree* for the final time period to subsequent BTM testing indicated that *PostPFree* provided a better estimate of herd-level antibody status than what can be achieved by relying solely on the previous test result.

INTRODUCTION

Bovine respiratory syncytial virus (BRSV) causes respiratory disease in cattle, and is an important agent in the bovine respiratory disease complex (BRD) in cattle worldwide (Paton et al., 1998; Luzzago et al., 2010; Ohlson et al., 2010; O'Neill et al., 2014). Infection caused by BRSV is most common in young animals, but the virus can infect cattle of all ages (Valarcher and Taylor, 2007). The virus is endemic in the Norwegian dairy population (Gulliksen et al., 2009; Klem et al., 2013). It causes increased use of antibiotics due to secondary bacterial infections, it has considerable negative impact on animal welfare and causes financial loss for the farmers (Larsen, 2000; Valarcher and Taylor, 2007). In 2016, a national control program against BRSV and bovine coronavirus (BCV) was launched in Norway, as the first country in the world. Participation is voluntary, and the program is conducted as a joint initiative amongst the producer organisations. In early 2016, bulk tank milk (BTM) was sampled from the majority of Norwegian dairy herds and analysed for BRSV and BCV antibodies. As part of a previous study, dairy herds in two counties on the West coast of Norway were also sampled three years before the national screening, and repeated BTM results were available for these herds. Currently, the control program is moving towards classification of herds based on individual samples (pooled samples of serum from young stock, or milk from primiparous cows), but results are not yet available for research purposes. BTM antibody testing was

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therefore used in the current study. A key strategy of the control program is to protect test negative herds by restrictions on livestock trade and thus limit direct contact between negative and positive herds. A negative herd test lasts for one year before retesting is required. This applies regardless of the degree of contact with other herds. In a previous Norwegian study, the dynamics of BRSV infection amongst herds were rapid, i.e. the elimination rate and introduction rate were high (Klem et al., 2013). Due to the constant risk of virus introduction, the assumption of a long lasting negative status is questionable for many herds. Several factors can affect the risk of change in status. Purchase of livestock is a well-known route of introduction of infectious agents, and herds that frequently purchase animals are likely at higher risk of seroconversion (Elvander, 1996). In addition to possible introduction of virus, purchase of antibody positive animals might also lead to a positive BTM test result. Besides purchase of livestock, previous studies have shown that location and herd size are important risk factors for antibody positivity (Ohlson et al., 2010; Toftaker et al., 2016). Regional differences in prevalence entails that the probability of introducing an infected animal through purchase depends on from where animals are purchased. Frössling et al. (2014) showed how the probability of introducing at least one infected animal through purchase varied with different between- and within-herd prevalences.

Documentation of freedom from different diseases has often been important at a national level, and the use of scenario-tree models has recently provided a more advanced and flexible approach to these calculations (Martin et al., 2007a). More et al. (2013) extended this methodology to calculations at the herd level. They included information on livestock trade along with test results in calculations of probability of freedom from Johne's disease in test negative herds in Ireland. In Norway, information on location of herds, herd size and livestock trade are available from central registries, and it was hypothesised that this information could be included along with test results to give an updated herd probability of antibody positivity, which would reflect the status of specific herds more accurately than previous BTM test results alone. Estimating a time-varying probability of freedom/infection could potentially form a better tool for risk assessment in livestock trade or provide the foundation for a more risk-based approach to sampling.

The aim of this study was to develop a framework for estimation of probability of freedom from BRSV antibodies (*PostPFree*) in specific herds over time based on information from bulk milk testing, geographic location and animal movement data, and to validate the herd-level estimates against subsequent BTM test results.

MATERIALS AND METHODS

Study area and study population

The study area was two neighbouring counties in western Norway, namely Sogn og Fjordane and Møre og Romsdal. The study period was from Jan 2013 to Mar 2016. However, BTM samples collected Dec 2012 were included as time period one, i.e. belonging to the first three months of 2013 (see definition of time period later). The total dataset consisted of herds located in the study region who either had at least one ingoing animal movement, or contributed at least one BTM sample during the study period. Information on herds without movements, or without a BTM sample was not available; hence, the total cattle population in the study region was not known. A flowchart was made to describe the different subsets of herds used for the different analyses.

Data sources

The Norwegian food safety authorities provided data on cattle movements from the Norwegian Livestock registry. In the current study, animal movements refers to movements where the animal changes owner, which is mandatory to report. Information about herd size was retrieved from the Norwegian dairy herd recording system (NDHRS), BTM test results were provided by the largest producer organisation, TINE SA, and information about location of herds (coordinates, EUREF89/WGS 1984 UTM-32) was provided by the Norwegian Agriculture Agency.

Sampling and analysis of BTM

As part of a cross-sectional risk factor study (Toftaker et al., 2016), BTM samples were collected in Dec 2012 to June 2013 from the majority of herds in the study area (n=1347 out of 1854 dairy herds in the study region at the time). Some of the test negative herds (n=275) were resampled the next year (Feb – Aug 2014). Finally, 1148 herds also had a BTM sample from March 2016, collected as part of the national BRSV/BCV control program. All BTM samples were collected by the driver in conjunction with milk collection and cooled at a temperature of 2-4°C until received at the laboratory (TINE Mastittlab Molde) where samples were frozen between −18 and −20°C until the time of analysis. The 2013 and 2014 samples were analysed in the Norwegian lab, whereas the 2016 samples were shipped overnight to the Enfer laboratory in Ireland (Enfer Scientific, Naas, Ireland).

BTM samples collected in 2012-2014 were tested for antibodies against BRSV using the SVANOVIR® BRSV-Ab. Samples were analysed following the manufacturer's instructions. In brief: The optical density (OD) reading of 450 nm was corrected by the subtraction of OD for the negative control antigen, and percent positivity (PP-value) was calculated as (corrected OD/positive control corrected OD) x 100. According to the test manual, the recommended cut-off value was sample positive >10 PP (Svanova 2017). From 2016, all samples were analysed with the new MVD-Enferplex BCV/BRSV multiplex, hereafter referred to as the multiplex. A panel of four different antigens (recombinant proteins and synthetic peptides) was used for detection of BRSV antibodies. A positive test response results in chemiluminescence, captured by an imaging system, and measured in relative light units (RLU) by the Quansys Q view software (v 1.5.4.7). Antigens were combined in a parallel reading, i.e. the test was considered positive when the RLU-value of at least one antigen was above the cut-off. The applied cut-off values for the four different antigens included in the test were: 2000 for BRSV-A, 4000 for BRSV-B, 7000 for BRSV-C and 1700 for BRSV-D. In a diagnostic test evaluation study, of the multiplex along with the SVANOVIR® BRSV-Ab for BTM, the Se for the multiplex was estimated at 0.940, and the Se of the SVANOVIR® BRSV-Ab was estimated at 0.998 (unpublished data).

Both tests detect antibodies and not the antigen itself. Consequently, in the present study the term "positive" is used when referring to animals, herds or regions as having BRSV antibodies. Furthermore, all input variables in the probability model relates to antibodies, hence, the calculated probabilities relate to presence of antibodies, and not necessarily infection or presence of the virus.

Animal movements

All recorded animal movements where the destination herd was located in the study area were included. Duplicate records, i.e. movements where animal ID, source county, destination

herd and movement date where identical, were reduced to single records (n=8237). Records of movements where the same animal was moved back and forth between the same two herds, or to two different recipient herds, on the same day, were omitted (n=179). Records where the source county or the source herd was missing, and could not be retrieved from other variables, were also omitted (n= 56). After editing, the dataset included records of 45208 movements to 1802 destination herds located in the study region.

Probability of freedom

The probability of freedom over time was calculated for all herds entering the study period with a negative BTM test result, and was updated periodically according to the chosen time period; every three months.

A methodology for estimating the probability of freedom from disease by use of multiple sources of data was presented by Martin et al. (2007a; 2007b). More et al. (2013) adapted this methodology to herd level calculations of probability of disease freedom. Furthermore, a methodology to identify herds with an increased probability of disease introduction due to animal trade was developed by Frössling et al. (2014). The framework presented here is based on a combination of concepts from the mentioned studies. The probability of freedom over time was calculated for each herd through the following Eq. (1-5):

First, the probability of introducing at least one positive animal, *PIntroTrade*, to the destination herd was calculated for each unique combination *sd* of source herd *s* and destination herd *d* for each time period as seen in Eq. (1).

$$PIntroTrade_{sd} = 1 - \ (1 - P(D+)_a)^n \quad (1)$$

where $P(D+)_a$ was the within-herd prevalence in the source herd, set to 0.5 (i.e. a 50-50 probability of positivity/freedom) for all herds, and *n* was the number of animals purchased from the source herd.

The total probability of introduction from all animal purchases within each time period *t* was calculated for each destination herd as seen in Eq. (2).

$$PIntroTrade_{all} = 1 - \ \prod(1 - (P(intro\ inf)_h \ \times \ P(D+)_h)) \quad (2)$$

where $P(D+)_h$ is the probability that the source herd is antibody positive at the herd level. As an estimate of $P(D+)_h$ the between-herd prevalence in the county of the source herd based on the national BTM screening was used.

As introduction of virus can happen not only through purchase of livestock, but also by indirect transfer, a factor for probability of indirect transmission *PIntroLocal* was included. This factor was estimated from the proportion of herds that went from negative at the first sampling (2013) to positive at the last sampling (2016), in the group that did not purchase animals. This was done separately for the two counties as it was known that the prevalence, and likely the infectious pressure, was higher in the north (Toftaker et al., 2016). Several studies have found an association between herd size and seropositivity (Norström et al., 2000; Solís-Calderón et al., 2007; Ohlson et al., 2010; Toftaker et al., 2016). In the study by Toftaker et al. (2016) the odds of testing positive increased with 12% across the inter quartile range of herd size. Based on this, the study herds were divided into two groups with median herd size as cut-off and assigned a value of *PIntroLocal* 12% higher in the "large" compared to the small herds.

In summary, this resulted in four categories of *PIntroLocal* based on herd size below or above median, and which county the herd was located in (north/south). The total probability of introduction through animal purchase and by indirect transmission for each time period *t* was then calculated as seen in Eq. (3).

$$PIntroTotal_t = 1 - \ (1 - PIntroTrade_t \ \times 1 - PIntroLocal) \quad (3)$$

The prior probability of infection at time *t, PriorPInf$_t$,* was estimated as seen in Eq. (4).

$$PriorPInf_t = PIntroTotal_t + \ PostPInf_{t-1} - PIntroTotal_t \ \times PostPInf_{t-1} \quad (4)$$

For the first time period (*t=1*), the prior probability of infection (*PriorPInf*) was set to 0.5, resembling testing a herd with completely unknown status i.e. no prior information on herd status available. *PriorPInf* was then calculated for each time period by taking the posterior probability of infection from the previous time period (*PostPInf$_{t-1}$*) and adding the probability of introduction during time period *t* calculated from Eq. 3, and adjusting for the possibility that it might already have been present but undetected at the end of the previous time period (*t-1*).

After each three month period, an updated probability of freedom (*PostPFree*) was calculated using Bayes theorem as described by Martin et al. (2007b) as seen in Eq. (5).

$$PostPFree = \frac{(1-PriorPInf)}{(1-PriorPInf \times TotalSe)} \quad (5)$$

The probability of infection (*PostPInf*) was then the complement to *PostPFree*. The change in *PostPFree* over time was visualized for two example herds in a line plot.

Sensitivity analysis

Due to the uncertainty of the local factor, a sensitivity analysis was performed, using 50% lower and 50% higher values of *PIntroLocal*, and assessing the effect on the outcome; *PostPFree*.

Model evaluation

To assess the usefulness of the developed method, the calculated estimates of *PostPFree* for the final 3 months were compared to the results from BTM testing in 2016. The herds were divided in two groups according to the dichotomized BTM results, and their ranking (according to the value of *PostPFree)* were compared using the Wilcoxon rank-sum test. The complement to *PostPFree*; *PostPInf,* is the probability of infection, or in this case antibody positivity. The accuracy of *PostPInf* was explored by treating it as a diagnostic test, using an arbitrary cut-off at 0.25 as well as the lowest calculated value and comparing it to the 2016 BTM result (used as gold standard). A smoothed lineplot of Se and Sp versus probability cut-off of *PostPInf* was made.

Software

All data management and analyses were performed using Stata (Stata SE/14; Stata Corp., College Station, TX).

RESULTS

Study population

The dataset consisted of 2432 beef and dairy herds located in Sogn og Fjordane and Møre og Romsdal counties. Of these, a BTM result from 2013 was available for 1347 herds, of which 725 where BRSV negative and used for probability of freedom calculations. Of the 1347 herds sampled in 2013, 1148 also had a BTM sample in 2016 of which 569 were initially negative and could be used for validation of *PostPFree*/*PostPInf*. For an overview of study sample and subsets of herds, see Fig. 1.

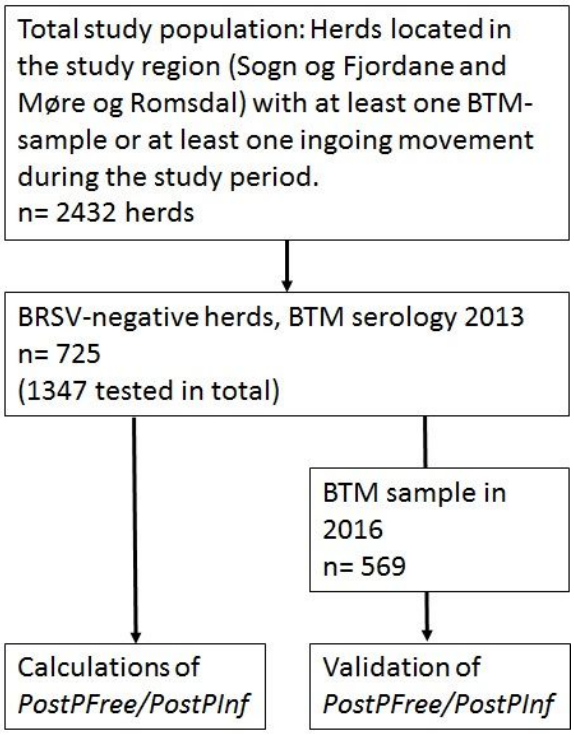

Fig. 3 Study sample and subsets of herds used in different parts of the calculations in a study estimating the probability of freedom from BRSV antibodies in dairy herds located in two counties in western Norway, during the period Jan 2013- Mar 2016.

BTM results

At the first sampling in 2013, 622 out of 1347 sampled herds were BRSV-antibody positive i.e. a proportion of test positive of 46.2% as previously reported (Toftaker et al., 2016). A few negative herds (n= 275) had follow up samples the next year of which 49 (17.8%) had seroconverted to BRSV. In March 2016 the national control program started, resulting in BTM samples from 1565 herds in the study area. In this latest screening, 688 herds (44.0%) were positive for BRSV antibodies. An overview of counts and proportions of test outcomes is presented in Table 1.

Table 1. Overview of BRSV antibody test result for BTM samples in 2013 and 2016 in a study calculating the probability of freedom from BRSV antibodies in dairy herds located in two counties in western Norway.

Year	BRSV+		BRSV-	
	n (%)		n (%)	
2013 n= 1347	622 (46.2)		725 (53.8)	
2016 n= 1565	688 (44.0)		877 (56.0)	
2013/2016	+/+	+/-	-/+	-/-
n= 1148	334 (38.0)	200 (17.4)	178 (15.5)	436 (38.0)

Local transmission factor

Of herds that did not buy animals during the study period (n=384), 104 herds were initially test negative for BRSV antibodies in each county. When retested in 2016, 21 (20%) of the initially negative herds had seroconverted in the southern county, and 36 (35%) in the northern county. After applying two different categories for herd size this resulted in a local transmission rate per (three month) time period of 0.015 for small herds in the southern county, 0.016 for large herds in the southern county, 0.025 for small herds in the northern county and 0.028 for large herds in the northern county.

Probability of freedom

The probability of freedom (*PostPFree*) after a negative test was initially high. In time period 12, the median *PostPFree* was 0.62, range 0-0.91. Purchase of animals greatly affected the *PostPFree*, resulting in different slopes for herds that purchased animals compared to closed herds, as shown by the example herds in Fig. 2. The distribution of *PostPFree* in time period 12 is shown by county in Fig. 3.

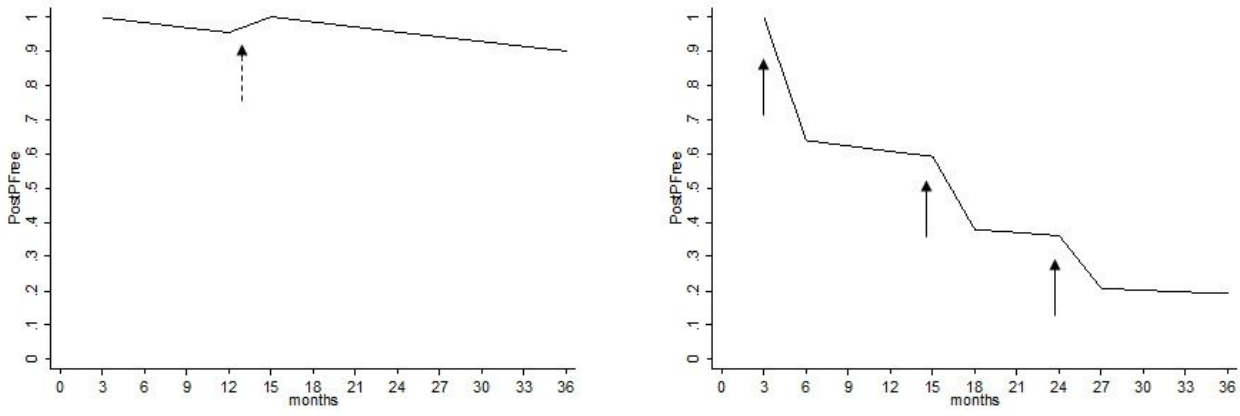

Fig. 4 Herd level probability of freedom (*PostPFree*) for BRSV antibodies over 36 months for two example herds both starting with a negative bulk tank milk (BTM) test. The herd to the left has no purchases, but a second BTM test indicated by a dashed arrow, whereas the herd to the right has purchased livestock on several occasions indicated by solid arrows Calculations were based on BTM antibody testing, herd location and animal movement data, and *PostPFree* was updated every three months.

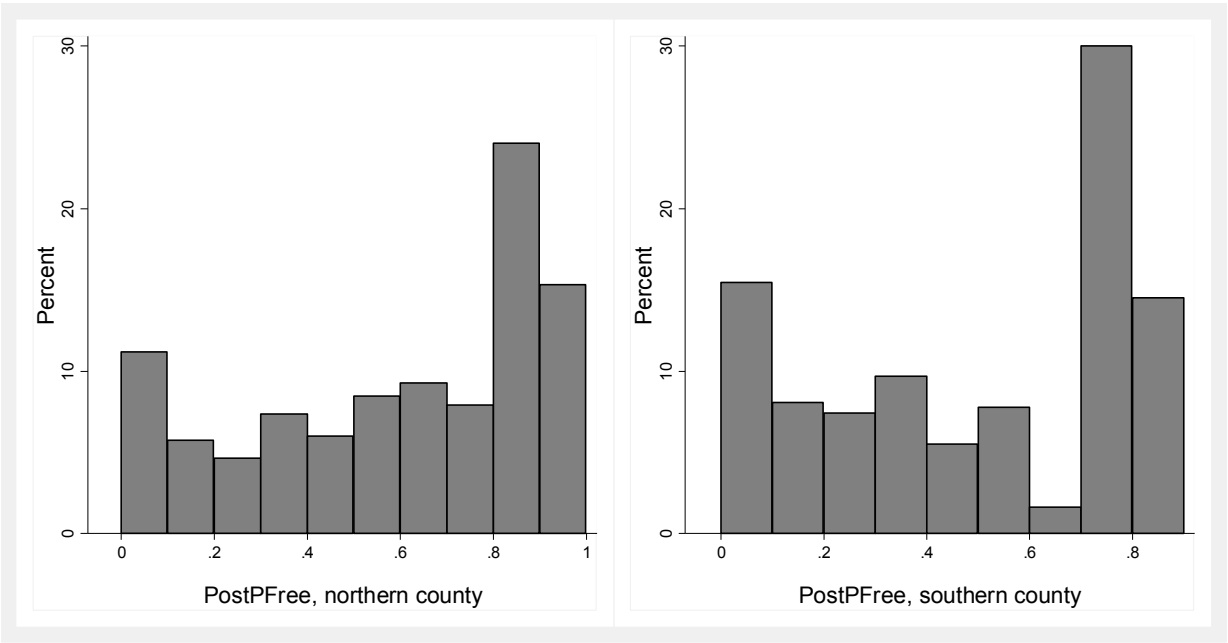

Fig. 5 The distribution of the probability of freedom (*PostPFree)* from BRSV antibodies in time period 12 for 720 Norwegian dairy herds that were initially BRSV-antibody negative based on bulk tank milk (BTM) testing conducted in 2013. Calculations of *PostPFree* were based on BTM antibody testing, herd location and animal movement data, and were updated periodically every three months. Time period 12 was the last three month time period before retesting of BTM (2016).

Sensitivity analysis

Reducing the value of *PIntroLocal* by 50% gave a mean increase in *PostPFree* of 10.6% (SD 4.6%), and increasing the value of *PIntroLocal* gave a mean decrease in *PostPFree* of 9.6% (SD 3.8%).

Model evaluation

The Wilcoxon Rank Sum test showed a significant ($p<0.001$) difference in *PostPFree* between the two groups; BTM positive and BTM negative herds in 2016. When assessing *PostPInf* as a diagnostic test, the Se decreased with increasing cut-off. At a cut-off for estimated probability of positivity > 0.25 (*PostPFree* < 0.75), the relative Se was 0.76. In a practical sense, a recommended retesting at this value would capture an estimated 76% of the "true" positive herds i.e. herds that are misclassified as negative based solely on the previous BTM test. No herds had *PostPInf* < 0.05 (*PostPFree* > 0.95) at the end of the study period, but at the lowest estimated value, *PostPInf* < 0.086, only two out of 15 herds (13%) were test positive. The Se and Sp of *PostPInf* is illustrated in Fig 4.

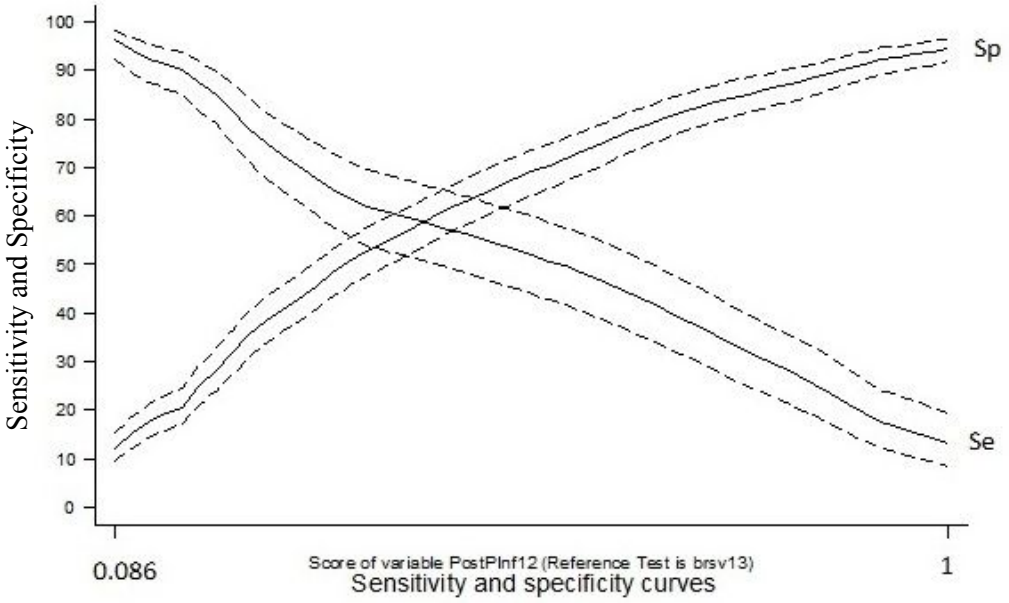

Fig. 4 Relative sensitivity and specificity of *PostPInf (1-PostPFree)* in time period 12 versus cut-off value, when the BTM antibody-test in time period 13 was used as gold standard. Calculations of *PostPFree* were based on BTM antibody testing, herd location and animal movement data, and were updated periodically every three months. Time period 12 was the last three month time period before retesting of BTM in time period 13.

DISCUSSION

This study shows that the *PostPFree* can be used as an updated measure of the herd level probability of freedom from BRSV antibodies. The *PostPFree* of a test negative herd was high immediately after sampling, reflecting the high sensitivity of the BTM test, but gradually decreased with time. Based on the calculations, the effect of the local factor was small compared to the effect of purchasing livestock, which had a large impact on the probability of freedom. The *PostPFree* was therefore very different in the study herds at the end of the study period depending on to which extent the individual herd had purchased animals. This confirmed that, in many cases, inferring a herd's current status from an old BTM sample is problematic. The reason for the low estimated *Pintro local* was that only 29% of the initially negative herds seroconverted during the study period, and even fewer among herds that did not purchase livestock (27%), which was the group used for estimation of the local factor. Klem et al. (2013) found a significantly higher introduction rate (42% in six months) in a previous Norwegian study. However, the latter study differ from the present in two important aspects: It used a random sample of herds from the national dairy population, and herd classification was based on serum samples of a group of young stock. The difference in introduction rates could therefore be due to regional differences in disease occurrence and dynamics, and/or it might simply reflect that BTM negative herds represent a low risk stratum of the population. A negative BTM test means the herd has likely been free of circulating virus for a long time, as animals produce antibodies for years after exposure to the virus (Klem et al., 2014). If a herd has managed to stay free of infection for many years, it seems likely that it will remain this way. The estimation of *PIntroLocal* in the present study was based on a small sample size, and support from literature were scarce. However, the sensitivity analysis showed that the change in output (*PostPFree*) was moderate when *PIntroLocal* was increased or decreased with 50%.

The framework used in the present study could be extended to application at a national scale and also encompass herd classification based on individual samples. This would include estimation of herd Se for the different types of sampling strategies, as described by More et al. (2013) for Johne's disease in Ireland and recently for *Salmonella* surveillance in Sweden (unpublished data). It can be assumed that herds negative on BTM differ from negative herds where classification is based on individual samples (milk or sera) from a group of individuals, and that *PIntroLocal* will have to be estimated for these other groups. Differences in prevalence and geographically dependent risk factors like herd density might mean there are important differences in *PIntroLocal* for different regions, hence difference in the importance of local transmission should be investigated for different regions as well. There might also be differences between categories of herds based on other factors, such as biosecurity level, production type, and herd size.

As mentioned, *PostPFree* relates to presence of antibodies and not necessarily presence of virus. Ideally, one would prefer to use a test detecting the antigen itself in order to achieve a herds "true state of infectivity"; however, this is demanding to do on a large scale, and antibody testing is commonly used (Hägglund et al., 2006; Ohlson et al., 2009; Beaudeau et al., 2010). Animal purchase might mean introducing an antibody positive animal and not necessarily introducing virus, thus the estimated *PostPFree* is likely lower than the true probability of freedom from circulating virus.

The *PriorPInf* was set to 0.5 for the first time period. This is a conservative estimate as it assumes no useful prior information about infection status (Martin et al., 2007b). However, the high Se of the BTM antibody tests will entail a high probability of freedom immediately after testing even if the prior probability is low. The model is therefore robust regarding choice of prior in this case.

PostPFree/PostPInf was validated by using the BTM result from 2016, comparing *PostPFree* in the test- positive and negative group. There was a significant difference between the groups, suggesting a benefit of using *PostPFree* instead of relying on the previous BTM result alone. When assessing *PostPInf* as a diagnostic test it was shown how different cut-off values could influence how many herds would be correctly classified. In a practical setting this predicts the expected proportion of truly positive herds that are picked up if retesting is recommended at a certain value of *PostPInf* (*PostPFree*), instead of relying on results from annual tests. If used on close to real time data, one could decide on a cut off, and have an alarm when *PostPFree* drops below this value. This could enable timely intervention and a more risk-based approach to sampling. In addition to test strategy purposes, the *PostPFree* could be used to classify herds in more than two categories, thus providing a more updated input for risk assessment prior to livestock purchase.

In conclusion, estimation of the probability of freedom for individual herds over time, based on the framework presented in this study, gave considerable variation in values among study herds even when they had equal starting points, i.e. negative test results. Validation against repeated BTM sampling indicated a benefit of using *PostPFree* for an updated probability of a herd's antibody status instead of relying solely on a previous BTM test result.

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RISK FACTOR ANALYSIS ON INTRODUCTION OF BVDV INTO PREVIOUSLY BVDV-FREE HERDS IN THE NETHERLANDS

A.M.B. VELDHUIS*, M.H. MARS, L. VAN DUIJN, P. WEVER AND G. VAN SCHAIK

SUMMARY

The Dutch cattle industry has the intention to evolve from a voluntary to a national control program for Bovine Viral Diarrhoea Virus (BVDV). The optimal design should include measures to minimise the risk of re-introduction. The aim of the study was to assess risk factors for re-introduction of BVDV in previously free Dutch cattle herds. The incidence of BVDV infections decreased from 7% in 2007 to 4% in 2016. The odds of a reintroduction increased with the number of non-BVDV free neighbouring herds, herd size and purchase of pregnant cows. The spatial risk factors had the strongest association with the risk of a BVD breakdown. Although BVD control in the Netherlands has been voluntary, an increasing number of herds obtained a BVDV free status. However, the fact that the presence of non-BVDV-free neighbouring cattle herds is an important risk factor stresses the need for national control efforts to further reduce the BVDV incidence.

INTRODUCTION

Since 1997, The Netherlands has a national voluntary program that aims at the control of Bovine Viral Diarrhoea Virus (BVDV) in cattle herds. The proportion of dairy and non-dairy cattle farms with a certified BVDV-free status is increasing, up to 37% of dairy farms and 6% of beef farms in 2016. Consequently, the prevalence of herds that are suspected of the presence of cattle that are persistently infected (PI) with BVDV is gradually declining from 19.4% in 2007 to 8.7% in 2015 for dairy herds and 31.5% to 14.5% for non-dairy herds (GD Animal Health (GD), 2016). Although, infection pressure may decline and free herds cannot purchase cattle from herds with a lower status unless tested, a small proportion of certified BVDV-free herds experience an outbreak. Currently, within the BVD program, the percentage of breakdowns is declining to about 4% in 2016; yet it is unknown which factors determine the risk of reinfection in previously BVDV-free herds. The cattle industry has the intention to start a national control program in which all herds are obliged to participate. The optimal design of the national control program should include measures to minimise the risk of reinfection with BVDV in previously free herds. Therefore, the aim of the study was to assess risk factors for re-introduction of BVDV in previously free Dutch cattle herds.

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MATERIALS AND METHODS

Study population

Herds obtain a BVDV-free status with an intake procedure that includes (i) whole-herd screening and removal of PI animals, (ii) testing of new-born calves for virus during the following 10 months and when a PI animal is detected, removal followed by again 10 months testing of new-born calves. Dairy herds can screen their lactating animals with a real-time PCR on a bulk milk sample. When virus is detected, all cattle have to be screened with an antigen ELISA (Ag-ELISA) in whole blood samples; otherwise only non-lactating cattle have to be screened with the Ag-ELISA. In beef herds, all cattle are screened with the Ag-ELISA. The BVDV-free status can be monitored by testing five randomly selected calves at 8-12 months of age with an antibody ELISA ('spot test') twice yearly. One seropositive animal in the spot test is considered a false positive result in terms of virus circulation at herd level. A herd will lose the BVDV-free status when at least three animals are seropositive in the spot test, which is considered an indication for virus circulation in the herd and will initiate further screening of individual cattle for virus. When two animals are seropositive, those are retested together with a new random sample of five calves 8-12 months old. Otherwise, the herd will retain the BVDV-free status. The BVDV-free status can also be monitored by testing all calves that are being raised on the farm for presence of BVDV with Ag-ELISA either in ear notch or in whole blood samples. A herd loses the BVDV-free status when virus is found in at least one animal in either blood or ear notch samples. Cattle from non-BVDV-free herds that are introduced into a BVDV-free farm must be tested for virus with an Ag-ELISA.

Selection of herds

In 2016, 85% of the herds participating in the BVDV-free program were dairy herds, of which 75% was BVDV-free and 25% was under investigation or in the intake process to become free. From the non-dairy herds in the program, 73% was BVDV-free and 28% was under investigation or in the intake process. For this study, all herds that were qualified as BVDV-free between September 2006 and June 2016 were selected. BVDV-free herds with a breakdown (i.e. at least one virus-positive animal or at least two seropositive animals detected) were classified as candidate-case herd. Only the first breakdown after becoming BVDV-free was selected. Any subsequent test result after the first breakdown was ignored, preventing duplicate case herds in the dataset. Herds without a breakdown were classified as candidate-control herd. Herds that left the program for unknown reasons, without having a breakdown, were assumed to be free until the moment they left the program. Certain (potential) risk factors were time-dependent, influenced by the decline in BVD prevalence over time in the Netherlands. It was therefore deemed important to assure an even distribution of case and control herds over the study period. For this reason, it was decided to match case and control herds by the moment they obtained their free status. The selection and matching of case and control herds was done in four steps.

First, all herds were grouped by the month and year in which the free status was obtained, and for each herd the cessation of the free period was determined based on spot test results (antibodies) or virus test results, depending on the type of monitoring. For herds without a breakdown, cessation of the free period was set at either (i) the month in which the herd had left the program or (ii) the end of the study period (June 2016; right-censoring).

Then, per time group, herds with a breakdown were selected as case herds. The remaining herds (candidate-controls) were randomly distributed over the case herds, provided that the

cessation of the free period of the candidate-control herd was at least six months after the breakdown of the case herd. In this way it was guaranteed that candidate-control herds were truly free at the moment of breakdown of the matching case herd. Case herds could have multiple matching control herds. Case herds without matching control herds were removed from the dataset. Certain (potential) risk factors were related to the period at risk prior to breakdown, yet control herds lacked a breakdown. Therefore, the moment of breakdown of the case herd was used to assign a corresponding (fictional) risk period to its matching control herds. For uniformity, this is also referred to as 'risk period' for control herds.

Risk factors

The risk factors that were deemed relevant based on literature and expert opinion and that were available in existing data sources are provided in Table 1. All data regarding risk factors were obtained from the Identification & Registration database with all cattle movements, a database comprising geographical coordinates of all livestock enterprises in the Netherlands and an in-house database regarding BVD certification statuses (GD). Data regarding purchased pregnant cows were available for a limited part of the study period (October 2006 to July 2013) and were considered missing when not available. It was assumed that the number of neighbouring cattle farms is fairly constant over time. A farm BVD certification status may change over time. For neighbouring herds, a fixed BVD status was used for the whole study period, based on the BVD certification status in November 2016. Neighbouring herds with an unknown BVDV status were classified as being non-BVDV-free. The month of breakdown (*Month*) was included to correct estimates for a changing risk of BVDV introduction (i.e. the decreasing BVD prevalence in time).

Data analysis

Statistical analyses were performed using STATA/SE version 14 software. Multivariable logistic regression analyses (the logistic procedure) were conducted at herd level to describe the relationship between potential risk factors and a breakdown in previously BVDV-free herds. All potential risk factors were included in the initial model, with the exception of the variables related to purchase. To prevent multicollinearity between explanatory variables, model results were once explored with purchase variable *Purch*, and once with variable *PregPurch*. Linear associations of continuous variables were checked by exploring the pattern of effect sizes of variables when they were transformed to evenly distributed percentile categories (<P25, P25-P50, P50-P75 and >P75). Interactions between herd size and purchase variables were tested. The goodness of fit of the logistic models was assessed using Pearson's goodness of fit test (estat gof).

RESULTS

Descriptive results

The proportion of herds with a BVD breakdown decreased from 7% in 2007/2008 to 4% in 2015/2016. A total of 5,486 herds were selected for analysis, of which 1,123 were classified as case herd. The mean number of control herds per case herd was 4 (median: 3). Case herds were on average 24 months certified BVDV-free before the breakdown (median: 20). Herd characteristics of case and control herds are presented in Table 2. The median number of cattle in case and control herds amounted to 151 (IQR 104-210) and 144 (IQR 94-198) heads,

respectively. Within a radius of one kilometre case herds had a median of 6 (IQR 3-9) neighbouring cattle herds and control herds 5 (IQR 3-8).

Table 1. Available risk factors to assess the association with introduction of BVDV in previously BVDV-free herds in the Netherlands between 2006 and 2016.

Variable	Description	Type[a]	Categories	Label
Time	Month of breakdown	Cont.	-	Month
Herd size	Mean total number of cattle present in the herd in the year of breakdown	Cat.	<P25 P25-P75 >P75	Hsize
Presence of sheep	Mixed herd with cattle and sheep	Bin.	Yes No	Sheep
Number of purchased cattle	Number of purchased cattle in the 24 months preceding the breakdown	Cont.	-	Purch
Number of purchased pregnant cows, by status of herd of origin	Number of purchased cattle in the 20 months preceding the breakdown, being pregnant at the time of purchase.	Cat.	- No purchase of pregnant cattle - Only purchase of pregnant cattle from BVDV-free herds - Purchase of at least part of the pregnant cattle from non-BVDV-free herds	PregPurch
Number of certified BVDV-free or unsuspected neighbouring dairy farms	Number of dairy farms within a radius of 1 km with a BVDV-free or BVDV-unsuspected status[c,d]	Cont.	-	NDairyFree
Number of non-BVDV-free neighbouring dairy farms	Number of dairy farms within a radius of 1 km without a BVDV-free or BVDV-unsuspected status[c]	Cont.	-	NDairyNonFree

Table 1. Cont'd.

Variable	Description	Type[a]	Categories	Label
Number of certified BVDV-free or unsuspected neighbouring non-dairy cattle farms[b]	Number of non-dairy farms within a radius of 1 km with a BVDV-free status	Cont.	-	NOtherFree
Number of non-BVDV-free neighbouring non-dairy cattle farms[b]	Number of non-dairy farms within a radius of 1 km without a BVDV-free status	Cont.	-	NOtherNonFree

[a]Continuous, categorical, or binary variables
[b]Beef suckler herds, veal herds, young stock raising herds, small-scale cattle farms and cattle traders.
[c]Based on the BVDV-status in November 2016.
[d]Alternative to the BVDV-free status, dairy herds can obtain a BVDV unsuspected status based on Ab-ELISA on bulk milk samples with quarterly intervals. A herd is granted the BVDV unsuspected status when the first bulk milk result is seronegative, this is then monitored by continuing the quarterly bulk milk testing.

Table 2. Characteristics of the Dutch cattle herds in the study to investigate risk factors for introduction of BVDV in previously BVDV-free cattle herds between 2006 and 2016

Herd characteristics	Case herds (n=1,123)	Control herds (n=4,363)
	%	%
Dairy herds	81.6	78.5
Herds with sheep	14.2	14.2
Herds without purchase of cattle in the 24 months prior to breakdown	48.6	47.7
Herds without purchase of pregnant cattle from non-BVDV-free herds in the 20 months prior to breakdown	80.4	84.2

Risk factors for breakdown

Two separate models were investigated; one including the purchase variable *Purch,* and one including the variable *PregPurch*. The latter was based on a subset of herds because data regarding purchased pregnant cattle where available only for a limited part of the study period.

Model with total number of purchased cattle: Results of the model are displayed in Table 3. The probability of a breakdown decreased per month during the study period (odds ratio (OR) 0.98; *p*-value = 0.000), which was in agreement with the decline in BVD prevalence in time in the Netherlands (GD Animal Health, 2016)). With each neighbouring non-BVDV-free dairy farm, the odds of a breakdown increased 1.12 fold (*p*-value = 0.000). With each neighbouring non-BVDV-free non-dairy farm, the odds on a breakdown increased 1.06 fold (*p*-value = 0.000). The number of BVDV-free neighbouring dairy farms and non-dairy farms was not associated with the probability of a breakdown, neither is the presence of sheep on the farm. Herd size is positively associated with the probability of a breakdown, with an OR of 1.33 (*p*-value = 0.002) fold increase in medium sized herds and a 1.59 (*p*-value = 0.000) fold increase

in large herds, compared to small herds. The number of purchased cattle during the 24 month-period prior to the breakdown was not associated with the probability of a breakdown.

Table 3. Results of multivariable logistic regression analysis of risk factors for a breakdown in BVDV-free herds, with risk factors and their categories, odds ratios (OR) 95% confidence intervals and significance *(p*-value) and model fit statistics (Pseudo R^2) (n=5,290 herds).

Variable	Category	OR	95% CI	*p*-value
Month	Continuous	0.98	0.98-0.99	0.000
NDairyFree	Continuous	1.03	0.95-1.07	0.203
NDairyNonFree	Continuous	1.12	1.10-1.21	0.000
NOtherFree	Continuous	0.90	0.78-1.03	0.130
NOtherNonFree	Continuous	1.06	1.03-1.08	0.000
Sheep	No	Ref.	-	
	Yes	1.09	0.89-1.32	0.402
Hsize	Small (< 96 cattle)	Ref.	-	
	Medium (96-200 cattle)	1.33	1.12-1.59	0.002
	Large (>200 cattle)	1.59	1.31-1.95	0.000
Purch	Continuous	1.00	1.00-1.00	0.444
Pseudo R^2 (McFadden)	0.047			

Model with purchase of pregnant cows: The effect of herd size on the probability of breakdown appeared to interact with the effect of purchase (of pregnant cows). Therefore, purchase was categorized in three categories (i.e. no purchase, purchase from BVDV free herds and purchase from non-BVDV-free herds) and combined with herd size in a three-by-three interaction term. Results of the model are displayed in Table 4. The effects of the herd size with purchase interaction term are displayed in three sections (A,B,C), each with one of the 'no purchase' categories as reference category. Duplicate comparisons are indicated by matching superscript. The probability of a breakdown decreases per month during the study period (OR 0.99; *p*-value = 0.000). With each neighbouring non-BVDV-free dairy farm, the odds of a breakdown increased 1.11 fold (*p*-value = 0.000). With each neighbouring non-BVDV-free non-dairy farm, the odds of a breakdown increased 1.06 fold (*p*-value = 0.000). The number of BVDV-free neighbouring dairy or non-dairy farms is not associated with the probability of a breakdown, neither is the presence of sheep on the farm. The purchase of pregnant cows from non-BVDV-free farms is associated with a 1.56 (*p*-value = 0.008) and 1.87 (*p*-value = 0.001) fold increase in the probability of a breakdown in medium and large herds, respectively, compared to small herds without purchase of pregnant cows (Table 4; section A). The purchase of pregnant cows from non-BVDV-free farms is associated with a 1.66 (*p*-value = 0.005) fold increase in the probability of a breakdown in large herds, compared to medium herds without purchase of pregnant cows (Table 4; section B). Herd size is positively associated with the probability of a breakdown. This became evident when comparing different groups of herds without purchase of pregnant cows. Large herds without purchase have a 1.49 (*p*-value = 0.006) and 1.31 (*p*-value = 0.026) fold increased probability of a breakdown compared to small and medium herds without purchase, respectively (Table 4; section A and B). Herd size is also a risk factor in herds that purchase pregnant cows from non-free herds, but not in herds that solely purchase cattle from BVDV-free herds (results not shown).

Table 4. Results of multivariable logistic regression analysis of risk factors for a breakdown in BVDV-free herds, with risk factors and their categories, odds ratios (OR) 95% confidence intervals and significance (*p*-value) and model fit statistics (Pseudo R^2) (n=3,043 herds). Number of observations per category is provided for interaction term Hsize * PregPurch.

Section	Variable	Category (number of herds)	OR	95% CI	*p*-value
	Month	Continuous	0.99	0.98-0.99	0.000
	NDairyFree	Continuous	1.02	0.96-1.09	0.443
	NDairyNonFree	Continuous	1.11	1.05-1.17	0.000
	NOtherFree	Continuous	0.88	0.74-1.04	0.135
	NOtherNonFree	Continuous	1.06	1.03-1.09	0.000
	Sheep Ref.= No	Yes	1.00	0.79-1.27	0.987
A	Hsize*PregPurch	Small – No purch. (644)	Ref.	-	-
		Small – Purch. Free (43)	0.91	0.40-2.10	0.798
		Small – Purch. Non-free (97)	0.75	0.43-1.33	0.327
		Medium – No purch. (1,240)	1.13	0.89-1.44	0.312
		Medium – Purch. Free (151)	1.05	0.67-1.65	0.825
		Medium – Purch. Non-free (275)	1.56	1.12-2.17	0.008
		Large – No purch. (517)	1.49	1.12-1.97	0.006[a]
		Large – Purch. Free (88)	0.90	0.50-1.61	0.726
		Large – Purch. Non-free (171)	1.87	1.28-2.74	0.001
B		Small – No purch. (644)	0.88	0.70-1.13	0.312
		Small – Purch. Free (43)	0.80	0.36-1.76	0.572
		Small – Purch. Non-free (97)	0.67	0.38-1.25	0.148
		Medium – No purch. (1,240)	Ref.	-	-
		Medium – Purch. Free (151)	0.93	0.61-1.43	0.740
		Medium – Purch. Non-free (275)	1.38	1.02-1.86	0.035
		Large – No purch. (517)	1.31	1.03-1.67	0.026[b]
		Large – Purch. Free (88)	0.80	0.45-1.40	0.429
		Large – Purch. Non-free (171)	1.66	1.16-2.36	0.005

Table 4. Cont'd

Section	Variable	Category (number of herds)	OR	95% CI	*p*-value
C		Small – No purch. (644)	0.67	0.51-0.89	0.006[a]
		Small – Purch. Free (43)	0.61	0.27-1.36	0.222
		Small – Purch. Non-free (97)	0.51	0.29-0.90	0.019
		Medium – No purch. (1,240)	0.76	0.60-0.97	0.026[b]
		Medium – Purch. Free (151)	0.71	0.45-1.11	0.133
		Medium – Purch. Non-free (275)	1.05	0.75-1.47	0.776
		Large – No purch. (517)	Ref.	-	-
		Large – Purch. Free (88)	0.61	0.33-1.09	0.090
		Large – Purch. Non-free (171)	1.26	0.86-1.85	0.237
	Pseudo R^2 (McFadden)	0.024			

[a,b]Identical (reversed) comparisons are indicated by matching superscripts.

DISCUSSION

This study showed that the risk of re-introduction of BVDV in free herds in the Netherlands has decreased from 2007 till 2016. This is supported by the decreasing prevalence of cattle herds with a recent BVDV infection (GD Animal Health, 2016). Although BVD control in the Netherlands was voluntary in the study period, an increasing number of herds obtained a BVDV-free status, which may have resulted in a decreasing infection pressure. However, the fact that the presence of non-BVDV-free neighbouring cattle herds is found to be a risk factor for a breakdown in a BVDV-free herd stresses the importance of national control efforts to further reduce the BVDV incidence.

The risk that the presence of a dairy herd neighbour poses was larger than the risk that the presence of a non-dairy herd neighbour poses (ORs of 1.12 and 1.06 respectively). The risk of (truly) BVDV infected neighbours is probably underestimated because in the model all herds with an unknown status for BVD were classified as non-BVDV-free, while in reality only about 20% of the dairy and 17% of the non-dairy herds with an unknown BVDV status truly have BVDV circulation (GD Animal Health, 2016). The true risk of having an infected neighbour (a herd with contiguous parcels) for a herd in which in the previous year no PI calves were born was determined for Ireland by Graham et al. (2016) who found odds ratios ranging from 1.07 to 3.02 for the number of neighbours with PI animals. The risk of purchasing cattle from herds with an unknown BVD status in the current study is probably also an underestimation of the true risk of purchasing from a BVD infected herd. In addition, herds with a BVDV free status have to test cattle for virus with an antigen ELISA if they are purchased from a herd with a lower BVDV status. This procedure seems effective given the result that the total number of purchased cattle in the two years preceding the breakdown was not a significant risk factor. Interestingly, purchase of pregnant cows did increase the risk of re-introduction of BVDV. A pregnant cow carrying a PI calf will not be detected with the antigen ELISA. In the voluntary BVDV control program pregnant cattle are not tested for BVDV-antibodies, which enable them to unknowingly reintroduce BVDV in a herd when their PI calf is born (Reardon et al., 2016). A national BVDV control program should include testing of pregnant cows for antibodies to mitigate this risk. The interaction term in the model between the categorical purchase variable

PregPurch and herd size indicated that the risk of purchase of pregnant cows increased with increasing herd size. This is expected to be due to the fact that larger herds in general purchase a larger number of cows than smaller herds, thus increasing the probability that at least one of these pregnant cows carries a PI calf.

Herd size was also a risk factor by itself: large cattle herds that had not purchased pregnant cattle had 1.5 higher odds of a breakdown than small herds that had not purchased pregnant cattle. When introduced in a herd, infectious diseases can more easily sustain in larger populations where there is a higher probability to have more susceptible cows, and for BVDV, more susceptible cows in the right stage of pregnancy to infect the foetus to become a PI calf. In this study, herd size is also likely to be indicative for other management factors that are not specified in this study and represent risk for re-introduction of BVDV, such as a larger number of professional visitors (e.g. veterinarians, AI technicians and traders) and more transport movements. Many studies have found herd size to be a risk factor for the presence of BVDV in a herd and only one investigated introduction of BVDV in a free herd. Presi et al. (2011) found herd size to be associated with the appearance of new cases of BVDV infection in the Swiss cattle population (herd size OR=1.01 for each additional animal).

In this study, the presence of sheep on the farm was not associated with the risk of re-introduction of BVDV. Other studies in Norway, Ireland and Scotland have investigated sheep as a risk factor for the presence of BVDV in cattle herds and also did not find an associated risk (Valle et al., 1999; Graham et al., 2013; Gates et al., 2013). Presence of sheep on the farm, as well as certain other explanatory variables such as the number of neighbouring farms and their BVDV-status, might have been biased as they could not be obtained for the herd-specific moment of breakdown (i.e. they were included for a fixed moment in time for all herds). This might have led to a certain weakening of the magnitude of risk factor effects (either positively or negatively).

Only few studies looked at risk factors for introduction of BVDV in free herds. One Dutch study described the introduction of BVDV in two dairy herds by purchase of pregnant heifers (Van Schaik et al., 1999). A study fairly similar to ours of Ersbøll et al. (2010) looked at neighbouring herds and herd size as risk factors for introduction of BVDV in free herds in Denmark. The model estimates of the Danish study are hard to compare with the estimates from the current study given the differences in the epidemiological situation and analytical approach. In Denmark, 13.3% of the free herds became infected in a 1.5 year study period, which was higher than the 4-7% incidence in the Netherlands. The median number of neighbours in Denmark was 6 with a median distance of 990 meters. The herd density seems similar to that in the Netherlands. However, in the Danish study only neighbours with a PI were included, while in the Dutch study neighbours with an unknown BVD status were included of which only a small part (17-20%) may have a PI. Notwithstanding, the Danish study also indicated that both herd size and the number of neighbours increased the odds of a breakdown for BVD.

Overall, the spatial risk factors had a stronger association with the risk of a BVD breakdown than the other risk factors. However, the low pseudo R^2 of the models indicated that much of the variation in the outcome variable was explained by other factors that were not included in the models. Nevertheless, this study showed that having neighbours with an unknown BVD status and purchase of pregnant cows increased the odds of a breakdown in a BVD-free herd.

Although BVD control in the Netherlands has been voluntary, an increasing number of herds obtained a BVDV-free status in the past decade. However, the fact that the presence of non-

BVDV-free neighbouring cattle herds is an important risk factor stresses the need for national control efforts to further reduce the BVDV incidence.

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AFRICAN SWINE FEVER IN DOMESTIC PIGS IN ESTONIA 2015–2017:

EPIDEMIOLOGICAL ANALYSIS OF OUTBREAKS

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SUMMARY

African swine fever (ASF) was first detected in Estonian wild boar population in September 2014. The first outbreaks in domestic pigs occurred in summer 2015. This paper summarises the results of epidemiological investigations of all 26 outbreaks that occurred during 2015 to 2017. On most of the affected farms, the first clinical signs were mild and not characteristic for ASF. The spread of the virus within farms has been slow and the contagiousness of the virus has been relatively low. Our findings suggests that virus introduction to the farms occurred via indirect transmission routes due to insufficient biosecurity. The presence of ASF virus in wild boar populations is the main factor facilitating infection of farms. All outbreaks occurred from June to September. The total herd incidence of outbreaks was similar across all three years, being 2.4% in 2015 and 2016, and 2.0% in 2017.

INTRODUCTION

Due to its serious impact on animal health and pig industry, African swine fever (ASF) is considered one of the most important and dangerous viral diseases of pigs and wild boar. The first case of ASF in Estonia was diagnosed in a wild boar found dead near the Latvian border at the beginning of September 2014. In the Latvian wild boar population, ASF was already present since June 2014 (OIE, 2014; Olševskis et al., 2016). In the following years, the virus spread through the Estonia's entire wild boar population, leaving only some islands free of infection. The first ASF outbreak in domestic pigs in Estonia occurred in July 2015 and was followed by 16 outbreaks over the following nine weeks. Six outbreaks were notified in 2016 and three in 2017. An overview of Estonian ASF outbreaks and cases is given in Table 1.

The aim of the present study was to retrospectively analyse the epidemiology of the disease in domestic pigs: in particular, the characteristics of the affected herds, the virus transmission and introduction pathways, the temporal and spatial outbreak patterns, and the herd incidence.

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Table 1. Number of detected ASF cases in wild boar and outbreaks in domestic pig herds in Estonia from 1st September 2014 to 31th December 2017.

	2014[a]		2015		2016		2017	
County	WB[b] cases	DP[c] outbreaks	WB cases	DP outbreaks	WB cases	DP outbreaks	WB cases	DP outbreaks
Harju	0	0	0	0	46	0	87	0
Hiiu	0	0	0	0	0	0	0	0
Ida-Viru	4	0	36	0	40	0	14	0
Jõgeva	0	0	60	2	192	3	15	0
Järva	0	0	102	1	117	1	9	0
Lääne	0	0	0	0	58	0	119	1
Lääne-Viru	0	0	91	1	198	1	64	0
Põlva	0	0	233	0	190	0	14	0
Pärnu	0	0	27	0	95	0	87	1
Rapla	0	0	6	0	203	0	90	0
Saare	0	0	0	0	98	1	305	1
Tartu	0	0	124	2	192	0	40	0
Valga	13	0	124	4	24	0	8	0
Viljandi	47	0	174	5	61	0	9	0
Võru	9	0	118	2	56	0	6	0
Total	73	0	1095	17	1570	6	867	3

[a]From 1st September

[b]Wild boar

[c]Domestic pig

MATERIALS AND METHODS

Epidemiological outbreak investigation

In all 26 affected farms, ASF epidemiological investigations were conducted either by the local veterinary officers or by the epidemiology team from the Estonian University of Life Sciences in compliance with Council Directive 2002/60/EC (European Commission, 2002). In principle, epidemiological enquiries dealt with (i) the length of time that the ASF virus may have existed on the holding before the disease was notified or suspected, (ii) the possible origin of the ASF virus at the holding and mode of introduction, (iii) the identification of other holdings at which pigs may have become infected from the same source.

Formal interviews using a structured questionnaire were conducted with farm managers, farm veterinarians and farm workers, focussing on farm management, herd data, animal movements, vehicle movements, feeding and bedding management, biosecurity measures and human activities, which might have facilitated virus introduction and spread. Furthermore, investigations were conducted focussing on clinical and pathological data and laboratory findings. Additional information including wild boar surveillance data, detailed data regarding laboratory analyses, and domestic pig population data were obtained from the Veterinary and

Food Board, the Veterinary and Food Laboratory– which is also the national laboratory for ASF, and the National Animal Register (NAR) of the Estonian Agricultural Registers.

All ASF outbreaks were confirmed by virus genome detection using real-time PCR (Tignon et al., 2011) in accordance with the EU diagnostic manual (European Commission, 2003). Tissue and blood samples were collected from all or selected dead or sick animals depending on the clinical course of the disease on the farm in question.

The epidemiological unit was defined as a group of pigs kept in one building or area (one out-door herd). Holdings were grouped into four size categories according to the number of pigs: 1–10 pigs (G1); 11–100 pigs (G2); 101–1000 pigs (G3); > 1000 pigs (G4). G1 holdings were classified as backyard or non-commercial farms where pigs are kept mainly for own consumption. G2, G3 and G4 holdings were classified as commercial farms.

The date and location of the closest wild boar case(s) to each outbreak farm were identified to evaluate the possible association between them. The Euclidean distance between each affected farm and the closest wild boar case was recorded.

The level of farm biosecurity was judged by a group of three experts as consensus judgment based on interview data and from observations made during farm visits. The first step involved evaluating farms based on their compliance to basic biosecurity requirements enforced by national legislation, and classifying them as compliant or non-compliant (Riigi Teataja, 1999; Riigi Teataja, 2004). In the second step the herds were further divided into five categories based on their biosecurity level as shown in Table 2.

The length of time that ASF virus may have existed on the farm before it was suspected (high-risk period: HRP) was estimated based on clinical and laboratory findings. In cases where antibody positive animals (detected by ELISA test) were found in an infected herd, it was concluded that the virus had been circulating in the herd for at least two weeks. In cases where sampled animals were only virus positive, the HRP was considered to be less than one week. However, if findings suggested that more than one infection cycle may have taken place, the HRP estimate was increased accordingly.

Statistical analysis

The yearly cumulative incidence of ASF outbreaks in domestic pigs was calculated across the different predefined herd size categories and farm types. The number of pig holdings (epidemiological units) recorded in the NAR in each respective group on 1st May every year (the date of yearly reporting by farmers) was used as the denominator in incidence calculations. Calculated incidences were used as incidence risk estimates and the 95% confidence intervals (Wilson score) were calculated to those using OpenEpi software (Dean et al., 2013). The statistical significance of differences in incidence risks was analysed using the Fisher exact test using the OpenEpi calculator; p values of ≤ 0.05 were considered significant.

RESULTS

Reporting and laboratory findings

ASF was immediately suspected on 12 out of the 26 farms, while on the other farms the first suspicion was feed poisoning (n = 7), erysipelas (n = 3), pneumonia (n = 3), salmonellosis

Table 2. Basic criteria for assessment of farm biosecurity level in ASF outbreak herds in Estonia, 2015-2017.

Criteria	Biosecurity level				
	Compliant				Non-compliant
	Very high	High	Moderate	Low	Very low
Indoor keeping	+	+	+	+	One or more requirements not fulfilled
Fence surrounding the farm territory	+	+	+	+	
Disinfection barriers at entry points to the farm territory for vehicles and humans	+	+	+	+/- [a]	
Disinfection barriers at entrances to farm buildings for humans and vehicles	+	+	+	+/-	
No swill and/or grass feeding	+	+	+	+	
No other farm and/or pet animals in a stable	+	+	+	+	
Adequate biosecurity procedures[b]	No deficiencies	1 deficiency	2 deficiencies	> 3 deficiencies	

[a]Partly fulfilled

[b]Functional infrastructure and procedures for disinfection; adequate procedures for entry of animals, humans, vehicles, equipment and materials; secure storage and handling of feed, and bedding material; existence of biosecurity plan

(n = 1) and heat or stress (n = 2). The reasons for reporting was sickness (n = 19) or death (n = 7) of one or several animals.

On all farms, PCR-positive animals were detected. In addition, on seven farms, animals with ASF virus specific antibodies were detected by ELISA. All antibody-positive animals were also PCR-positive. The estimated HRP varied from seven to 20 days with a median of 11 days (Fig. 1).

Characteristics of affected farms

The number of outbreaks across different types and sizes of farm is shown in Table 3.

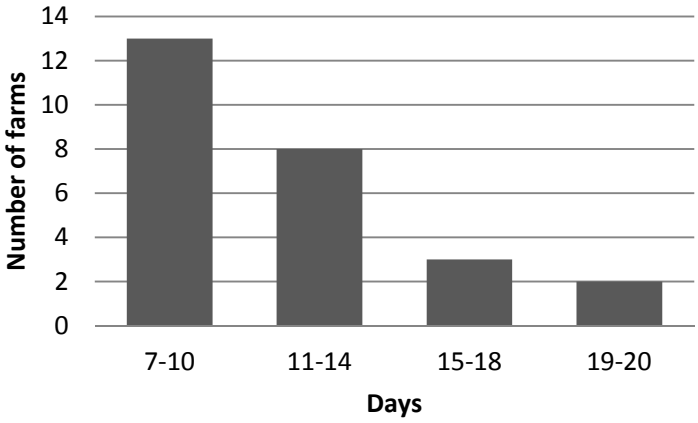

Fig.1 Length of estimated high-risk period in 26 pig farms affected by ASF in Estonia, 2015-2017.

Table 3. Distribution of Estonian ASF positive domestic pig farms across herd type and size, 2015–2017.

	Herd size (no. of pigs)				
Production type	G1 (1–10)	G2 (11–100)	G3 (101–1000)	G4 (> 1000)	Total
Multiplier	0	0	1	2	3
Farrow-to-finish	1	1	3[a]	5	10
Fattening	7	0	1	5	13
Total	8	1	5	12	26

[a]Two herds with crosses of wild boar and domestic pigs (one kept outdoors), and one organic pig farm

Twenty-four outbreaks were classified as primary outbreaks while two outbreaks were considered to be secondary outbreaks due to close contact with infected herds (common ownership and movements of farm workers, vehicles and equipment between farms). There was no movement of animals between these connected outbreak farms during the high-risk period.

Clinical signs and virus spread within farms

A summary of recorded clinical signs in pigs on affected farms is given in Table 4. The first clinical signs in pigs were often mild and not characteristic of ASF. Cases of severe courses of the disease were recorded on 13 farms. On 9 out of 12 farms where sows were kept, morbidity occurred first among pregnant or nursing sows. Skin haemorrhages or cyanosis were reported in pigs on 11 farms, often occurring in a few animals only.

Probable routes of virus entry into farms and biosecurity level of the outbreak farms

On all 26 outbreak farms, the virus was most likely to have been introduced by an indirect transmission pathway. On two farms (one commercial outdoor herd and one non-commercial herd with an outdoor walking area), direct contact (through fence) with infected wild boar could not be completely excluded. On eight non-commercial farms with no or very low biosecurity, virus introduction might have occurred via several pathways, e.g. contaminated feed, grass,

Table 4. Clinical signs in pigs recorded on 26 ASF outbreak farms in Estonia, 2015-2017.

Clinical manifestation	No. of farms
Loss of appetite	19
Listlessness	19
Sudden death without prior symptoms	14
Skin haemorrhages or cyanosis	11
Fever[a]	10
Recumbence	10
Incoordination	7
Abortions	5
Respiratory disorders	5
Other[b]	5

[a]On six farms fever was not detected; on ten farms temperature was not measured

[b]Vomiting (n = 2); decrease in milk yield of sows (n = 1); diarrhoea (n = 1); blood in urine (n = 1)

Table 5. Most probable pathways of ASFV introduction to commercial pig farms in Estonia, 2015–2017.

	Herd size category (no. of pigs)			
Introduction pathways	G2 (11–100)	G3 (101–1000)	G4 (> 1000)	Total
Multiple errors in execution of biosecurity procedures	1	0	4	5
Inadequate disinfection of vehicles	0	0	2	2
Minor errors in execution of biosecurity procedures	0	0	2	2
Movement of people (secondary outbreak)	0	1	1	2
Contamination of cereal feed during storage or processing	0	3	2	5
Feeding of grass to pigs	0	1	0	1
Contamination of bedding material	0	0	1	1
Total	1	5	12	18

clothing, vehicles, other farm animals or pets on the farm, and kitchen waste. The cause of virus introduction for these herds was defined as “lack of/insufficient biosecurity measures”.

For commercial herds, possible pathways of virus introduction were identified by the epidemiology team more specifically and are presented in Table 5. The biosecurity measures required by legislation as described in Table 2, at least at a minimum level, were in place for 46% (n = 12) of outbreak herds. In 35% (n = 9), the measures were implemented at least at a

moderate level, and 12% (n = 3) of outbreak herds had a high or very high biosecurity level. The biosecurity levels of affected farms are shown in Table 6.

Table 6. Biosecurity levels of ASF outbreak farms in Estonia, 2015–2017.

Herd size category (no. of pigs)	Very high	High	Moderate	Low	Very low
G1 (1–10)	0	0	0	1	7
G2 (11–100)	0	0	0	0	1
G3 (101–1000)	0	0	1	0	4
G4 (> 1000)	2	1	5	2	2
Total	2	1	6	3	14

The biosecurity level on all eight non-commercial (G1) farms was low or very low. On commercial farms (G2, G3, G4), the level was higher in general. The biosecurity level of seven (38%) commercial farms was estimated as very low; five because of the absence of a fence around the farm territory, one because of lack of a disinfection barrier at the entrance to the territory and one because of outdoor keeping.

Herd incidence

The observed cumulative herd incidence (incidence risk) of outbreaks in different years is presented by herd-size group in Table 7. In 2015, the herd incidence risk was significantly higher ($p < 0.05$) on commercial (G2, G3, G4) farms compared to non-commercial, backyard (G1) farms. In 2016, the herd incidence risk was not significantly different between these two groups ($p = 0.279$). In 2017, ASF was reported only in commercial herds. The total herd incidence risk across all herds did not differ significantly between years.

Spatial and temporal distribution of outbreak farms

The geographical locations of outbreak farms have changed during the epidemic. As shown in Fig. 2, domestic pig outbreaks have appeared in those areas where ASF virus was circulating actively in the wild boar population.

All ASF outbreaks were detected during the warmest period of the year, between June and September. Most of the outbreaks (81%) were detected in July and August (See Fig. 3).

Distance of wild boar cases from outbreak farms

Of 26 outbreaks, 23 occurred in regions where the disease was also present in the wild boar population (within a radius of 15 km from the affected farm). The distances between the outbreak farm and the nearest case of ASF in wild boar are shown in Fig. 4.

DISCUSSION

The ASF outbreaks on domestic pig farms were generally reported at a relatively early stage, during the first week after the appearance of clinical signs. This is fairly certain because no convalescent (antibody ELISA positive) animals were found. In two cases reporting was

Table 7. Number of ASF outbreaks and apparent herd incidence risk in Estonian domestic pig herds between 2015 and 2017.

	Year		
Herd category	2015	2016	2017
Commercial farms G2, G3, G4 (no. of herds)	212	152	128
Number of ASF outbreaks	13	2	3
ASF herd incidence risk	6.1%	1.3%	2.3%
95% confidence intervals of incidence risk	3.6..10.2%	0.4..4.7%	0.8..6.7%
Non-commercial farms G1 (no. of herds)	487	90	25
Number of ASF outbreaks	4	4	0
ASF herd incidence risk	0.8%	4.4%	0.0%
95% confidence intervals of incidence risk	0.3..2.1%	1.1..10.9%	0..13.3%
Total (no. of herds)	699	242	153
Number of ASF outbreaks	17	6	3
ASF herd incidence risk	2.4%	2.5%	2.0%
95% confidence intervals of incidence risk	1.5..3.9%	1.1..5.3%	0.7..5.6%

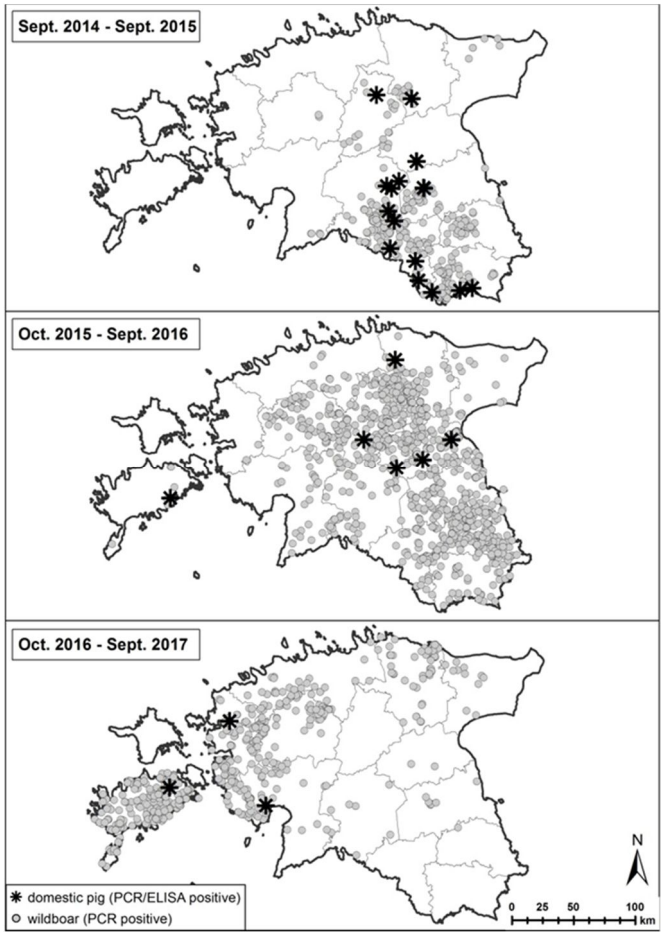

Fig.2 Location of ASF domestic pig outbreak farms and virus positive wild boar cases in Estonia in 2015, 2016 and 2017.

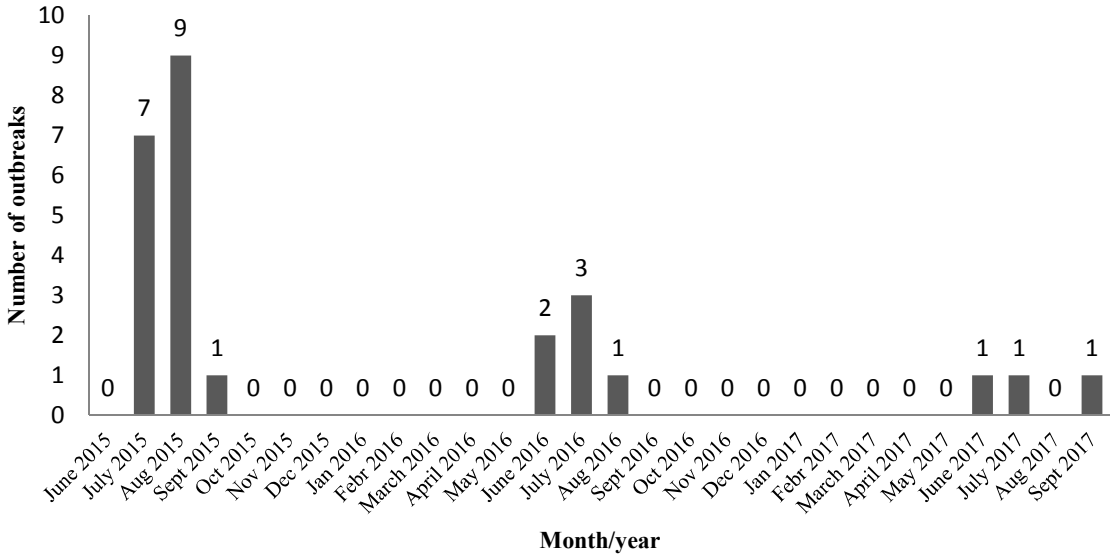

Fig.3 Occurrence of ASF outbreaks in Estonia from June 2015 to September 2017.

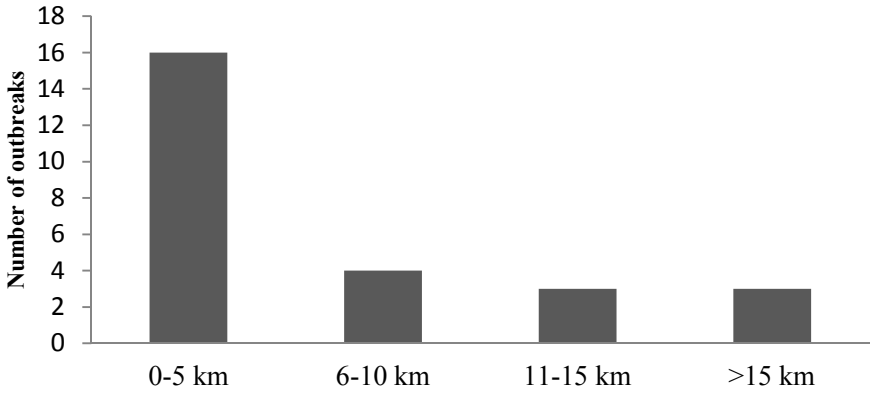

Fig.4 The distance between the closest tested ASF positive wild boar case and domestic pig outbreak farms in Estonia, 2015-2017.

delayed for about two weeks. In these herds, ASF antibody ELISA positive animals were also detected. However, all these animals were PCR-positive as well, which indicates that the virus had not been present in the herd for more than 3 weeks. The speed of reporting was not dependent on whether the herd was commercial or not. The two outbreaks with delayed reporting were both commercial herds.

Although ASF is described as a severe, haemorrhagic disease that causes up to 100% morbidity in naive pig herds and can result in very high mortality (Sánchez-Vizcaíno et al., 2009; Costard et al., 2013), ASF cases with mild clinical signs were often found and the haemorrhagic form seldom observed in field conditions. This can be explained by the relatively early detection of outbreaks, as most of them were reported within 7 days of the first observation of disease signs. Severe clinical courses and higher morbidity were seen in pregnant or nursing sows, or in the case of longer virus circulation on a farm.

In more than half of the outbreaks, diseases other than ASF were suspected. This can be mainly explained by non-typical signs of ASF at the beginning of the epidemic, particularly due to the lack of characteristic pathological post mortem signs (data not presented).

The outbreaks occurred in herds of all production types and size categories. Half of the outbreaks occurred in fattening herds and half in multiplier and farrow-to-finish herds. The proportion of outbreaks in herds with breeding animals (50%) is higher than the proportion of these herds in the general population (28%). This may be explained by the differences in management of breeding pigs and growers and fatteners (more human interaction with breeding pigs). On the other hand, pregnant and nursing sows may be more susceptible to the virus due to probably more compromised immune system function, while small doses of the virus might be able to initiate the infection.

The number of outbreaks in commercial herds exceeds the number of outbreaks in backyard farms. This can partly be explained by the rapid reduction of backyard holdings due to strict biosecurity requirements, which are equal to all pig farms. This brought the number of backyard pig farms down from 696 in 2014, to 25 by 2017. On the other hand, it may indicate that large commercial farms are more exposed to the virus due to more frequent and intensive contact with the external environment through movement of people and vehicles.

The spread of the virus within affected herds was generally slow. Even in affected pens, some pigs were still ASFV-negative at the time of reporting, and in most of the outbreaks the infection was detected in one unit or even in one pen. In conclusion, the contagiousness of ASFV could not be classified as high, meaning that mortality and morbidity figures remained low, even in the case of larger herds (data not presented). However, the case fatality rate was high, as affected pigs died 1–5 days after the appearance of the first clinical signs; this means that an ASF epidemic may result in high mortality if there is enough time for the virus to spread within the herd.

Based on the collected epidemiological information, the introduction of the virus into domestic pig herds is likely to have occurred mainly by indirect transmission routes. None of the outbreaks could be linked to the introduction of infected pigs. Direct contact with potentially infected wild boar could not be completely excluded in two herds – one outdoor farm of crosses with double fencing, and one organic farm using a single fence with walking area attached to the barn. However, even in these herds, direct contact was considered unlikely.

Feeding of contaminated swill has been generally presumed as being one of the main risk factors for indirect transmission of ASF (FAO, 2013; Gogin et al., 2013). In Estonia, the feeding of swill to pigs is illegal and could be excluded as a route of virus introduction on all affected commercial farms. On backyard farms, the feeding of kitchens leftovers could not be excluded. However, swill feeding was not considered to be one of the main possible routes of introduction, as the owners mainly used pig meat from their own pigs. Introduction of the virus to the farms with purchased meat products (hams, sausages etc.) from local shops would assume hidden circulation of the virus or contamination of imported products. This was considered unlikely. According to the interview results none of the farmers or farm workers had contacts to affected non-EU countries, thus the introduction of contaminated pig meat or products from these countries was also considered unlikely. Another possible source of infection is contaminated wild boar meat. Limited circulation and use of uncontrolled wild boar meat cannot be excluded in Estonia. However, evidence of the use of wild boar meat in affected backyard herds could not be established except for one case, where the owner was a hunter. Thus, most likely, the virus has entered the affected herds by means of contaminated fomites –

clothing, vehicles, feed and bedding material – due to inadequate biosecurity measures or errors in the implementation of these measures.

For most outbreaks there was no single obvious cause or event that could be linked with the introduction of the virus. In most affected backyard farms there were several biosecurity gaps at the time (e.g. lack of functional disinfection barriers, no separation of inside and outside zones, pet access or housing other farm animals together with pigs, feeding of grass to pigs, unsafe storage of bedding material and feed etc.). It is difficult to single out one particular cause. In commercial herds that followed relatively high biosecurity protocols, the route of virus introduction was difficult to trace. Seemingly, minor errors in the implementation of (generally adequate) disinfection procedures must have led to the introduction of the virus.

The majority of outbreaks occurred on farms with either a low or very low biosecurity level. However, looking at commercial farms separately, it appears that those farms with at least a moderate biosecurity level have experienced outbreaks to the same extent as those with low and very low biosecurity levels. It is generally assumed that low biosecurity level farms are at higher risk of introduction of infections. Based on available data, it was not possible to estimate whether herds with a low biosecurity level have been at higher risk or not, as information about the distribution of biosecurity levels for the whole population is lacking. However, assuming that the biosecurity level is in general higher on commercial farms than on backyard farms, the data found here on herd incidence do not support the general opinion that a higher biosecurity level ensures a lower risk of ASF introduction (see below). This may mean that the biosecurity measures applied so far (physical and disinfection barriers) are not fully effective in protecting against the incursion of ASF virus.

The herd incidence risk estimates are dependent on the accuracy of reporting. The observed herd incidence risk in non-commercial herds (backyard farms) did not differ significantly from the incidence risk in commercial herds (in 2016), or was higher in the group of commercial farms (2015 and 2017). One may question whether the reporting in the group of backyard farms was as good as for commercial farms or not. Considering the availability of veterinary services in Estonia (there are veterinarians available for every animal keeper), and the usual habits of smallholders in cases when their animals are sick, we would assume, at worst, only a slightly lower level of reporting in backyard herds compared to commercial farms. The surveillance in herds located in restriction zones (areas where infection in wild boars or domestic pigs has been detected) hasn't revealed any case of undetected infection in domestic pigs (data not shown).

The observed herd incidence risk in commercial herds decreased significantly in 2016 and 2017 from 2015. This is likely the result of improvements of biosecurity measures on farms, and more stringent surveillance by the veterinary authorities regarding the fulfilment of legal requirements on biosecurity. Interestingly, the total herd incidence across both groups of herds did not change significantly. However, it may be expected that there was some reporting bias for the group of backyard herds in 2015.

The occurrence of domestic pig outbreaks has been clearly associated with occurrence of ASF in wild boar. The vast majority of outbreaks in domestic pigs occurred in areas where ASF had been found in wild boar prior to detection of the virus in domestic herds. In 23 outbreaks, the virus had been circulating among wild boar within a radius of 15 km from the affected farm, and in 16 outbreaks, within a radius of 5 km from the affected farm. On the island of Saaremaa, the infection was first discovered in a domestic pig herd. However, over the following couple of days after the reporting of the case in domestic pigs, two infected wild boar carcases were found 3 km and 10 km respectively from the outbreak farm. The age of

these carcases indicates that the virus was present in the wild boar population for some time before the outbreak in domestic pigs occurred. The occurrence of outbreaks in domestic pigs seems to be associated with the intensity of the infection in the wild boar population – the outbreaks occur in areas where there are more virus-positive (as detected by PCR) cases in wild boar.

The introduction of ASF virus into domestic pig herds has been strictly seasonal in Estonia and associated with the warmest period of the year – June to September. Most of the outbreaks (81%) were detected in July and August. A similar seasonality trend has also been observed in other infected EU countries (Olševskis et al., 2016; EFSA, 2017). One explanation for this seasonality might be that during summer months the contact between farms (people and vehicles) and the wild boar in the surrounding environment is much more frequent because of the seasonal field work. The high-risk period for introduction of the virus into domestic herds coincides with the harvest period, when wild boar also move to feed in the fields. This is also the period when the wild boar density is highest (period after breeding season), and additionally, the number of infected wild boar is also at its highest, which indicates the infection pressure. All these factors may increase the probability of transmission via contaminated fomites.

The results of this study suggest that the presence of ASF virus in wild boar populations is the main risk for domestic pig farms to become infected. Farms of all sizes and types are at risk, including large commercial farms operating at a high biosecurity level. Farms with breeding animals seem to be at higher risk of becoming infected. Despite the high virulence of the circulating virus strain, the clinical manifestation of the disease has initially been unspecific and mild in most herds. The spread of the virus within farms has been slow and the contagiousness of the virus has been relatively low.

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ANTIMICROBIAL USE

QUANTITATIVE AND QUALITATIVE ASSESSMENT OF ANTIMICROBIAL TREATMENT INCIDENCE IN A CROSS-SECTIONAL STUDY OF EUROPEAN BROILER FARMS

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SUMMARY

With antimicrobial usage (AMU) being the most important driver of antimicrobial resistance (AMR) and a large share of veterinary AMU being related to the broiler industry, better insights into AMU in broilers are needed. Therefore, the aim of this study was to assess AMU in broilers at farm level in eight European countries. Data were collected from 161 farms to quantify AMU, using treatment incidence as the standardized unit of measurement. The median farm treated its broilers during 8% of the rearing period and 24% of farms already set up treatment on day one of production. Polymyxins, aminopenicillins and fluoroquinolones were the most frequently used antimicrobials with 26%, 26% and 16% of total AMU, respectively. Large variation in AMU in terms of amount, age of treatment and antimicrobial class was observed both within and between countries. This suggests that in many countries there is still substantial room for improvement in the amount and type of treatments administered.

INTRODUCTION

Antimicrobials play a pivotal role in modern medicine. However, due to the excessive exposure of bacteria to antimicrobials, more and more bacterial species have developed mechanisms that make them resistant to these drugs. Together with a fall back in the development of new medicines, bacterial resistance is threatening to bring modern medicine back into a pre-antibiotic era. This has led to the recognition by the World Health Organization (WHO) of antimicrobial resistance (AMR) as one of the major threats to public and animal health (Cars et al., 2008).

Antimicrobial usage (AMU) has been defined as the strongest driver for the selection of AMR, both in human and veterinary medicine. However, the majority of global AMU is related to animal husbandry (Landers et al., 2012). In particular, the broiler industry is responsible for a large share of the animal related AMU. With 13,720,000 tons of poultry meat produced each year within the European Union (EU), the broiler industry is the second biggest meat producing industry (Eurostat, 2016). In addition, it is also a very intensive animal production system. One

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house can contain up to a 100,000 broilers, which makes disease prevention a priority. Furthermore, almost all antimicrobials are administered at the flock level through drinking water or through medicated feed as this is the most convenient and cheapest way. This type of mass medication leads to larger amounts of antimicrobials being used, compared to individual administration where only the sick animals are treated (Landoni & Albarellos, 2015).

To control the emerging threat of AMR, scientific research proposes appropriate and detailed monitoring of AMU on supranational, species and farm level (Chantziaras et al., 2017). In 2009, ESVAC (European Surveillance of Veterinary Antimicrobial Consumption) was founded to collect and report data on AMU in the member states of the European Union. This project has succeeded in the collection of harmonized data concerning AMU in EU member states and a few other European countries, based on sales data. The results have repeatedly elucidated huge differences between countries in the amount of antimicrobials sold (EMA, 2015a). However, the used indicator does not take differences in dosage between antimicrobials into account, nor does it allow for monitoring of AMU by species or at farm level. In addition, reports on AMU based on sales figures remain a crude measurement (Sjölund et al., 2016). This makes it difficult to draw valid conclusions on usage profiles and differences based upon the ESVAC data. Avoiding approximations and corrections is only possible when collecting the actual use of antimicrobials at the farm itself (Ferreira & Staerk, 2017). However, studies collecting AMU data by species and at farm level, in several countries simultaneously, using the same protocols, are laborious and time-consuming and to the authors' knowledge inexistent for the broiler industry. Therefore, the aim of this study was to quantify AMU in broilers at farm level in eight European countries in a standardised manner to get better insights into AMU in broilers

MATERIALS AND METHODS

Study sample

In this cross-sectional survey, data concerning antimicrobial usage (AMU) were collected at 20 farms (21 in Belgium) in each of the eight participating countries: Belgium, Bulgaria, Denmark, Germany, Italy, Poland, Spain and The Netherlands. Farms were selected from national identification and registration databases and needed to meet certain selection criteria. Only conventional broiler farms were allowed, meaning that the farms had an intended slaughter age not higher than 50 days, no slow growing breeds were used (growth rate less than 55g/day) and stocking density was not lower than 10 birds/m2. In addition, all farms needed to have an all-in/all-out production with a thinning procedure allowed from day 30 onwards. Eligible farms, willing to collaborate in the study, were randomly selected. Full random selection was not always possible, leading to deviations from the protocol as described in the supplementary material of Munk et al. (2017).

Data collection

All farm visits took place between May 2014 and June 2016. During farm visits a questionnaire, developed within the EFFORT consortium, was completed. On the one hand, all antimicrobials administered in one production cycle were registered in detail for the sampled broiler houses, and for each course of treatment. These data were the group treatment data, covering AMU in one house during one production round. On the other hand, a purchased products dataset was set up. These data were based on the purchased antimicrobials in the year preceding the farm visit and were collected through invoices or registers. This dataset covered

the purchased antimicrobials for the whole farm over the period of one year. The full questionnaire can be provided upon request.

Quantification of antimicrobial consumption

The necessary data from the questionnaires were put into EpiData version 3.1 software by the researchers completing the farm visits (EpiData Association, Denmark). Afterwards a first data quality control was performed (e.g. missing values, matching farm identifications) and files were corrected if necessary. Finally, EpiData files were converted to Microsoft Excel for a more in-depth data quality control (e.g. units, outliers).

$$\text{TI} = \frac{\text{Total amount of antimicrobials administered or purchased}}{\text{DDDvet or DCDvet or UDDvet x number of days at risk x kg animal at risk}} \text{ x 100 broilers at risk} \quad (1)$$

AMU was quantified in a standardized manner using treatment incidence (TI) as described for broilers by Persoons et al. (2012). Treatment incidence is expressed as the percentage of broilers being treated with antimicrobials each day, or the percentage of their life span that the broilers are being treated. In the denominator of the treatment incidence formula (Eq. (1)), three different parameters can be used: Defined Daily Dose (DDDvet), Defined Course Dose (DCDvet) and Used Daily Dose (UDDvet). These parameters are used to take into account differences in dosage between products. DDDvet and DCDvet reflect the average maintenance dose of a drug per day per kg broiler and per treatment course per kg broiler respectively, in relation to its main indication. Used daily dose represents the actual dosage used on the farm itself. Each active substance has its own DDDvet and DCDvet per unique combination of administration route and species. For antimicrobials registered for broilers, most of these values have been defined by ESVAC (EMA, 2016). Combination products, where the dose of one or both of the active substances are substantially different from the single active substance products, were assigned different DDDvet and DCDvet values. In all other cases of combination products, the assigned values were the same as the single active substance antimicrobials (EMA, 2016).

The different types of TI were calculated separately for the data of the group treatments (TI_{DDDvet}^{*}, TI_{DCDvet}^{*}, and TI_{UDDvet}^{*}) and a second time based on the data of purchased products over the period of one year (TI_{DDDvet}^{**}, TI_{DCDvet}^{**}). Afterwards TI was determined on farm level ($TI_{DDDvetF}^{*/**}$, $TI_{DCDvetF}^{*/**}$, $TI_{UDDvetF}^{*}$), by summing up TI's from all the treatments within a farm, and this for each type of TI. Additionally, TI at the country level was determined by calculating the average $TI_{DDDvetF}$ of the sampled farms within a country. Treatment incidence was also summed for each antimicrobial class separately, allowing for a comparison of AMU per class in total and between different farms or countries.

Statistical analysis

All calculations of TI were done by using R version 3.4.0. software (https://cran.r-project.org). Descriptive statistics were performed in MS Excel 2016. Differences between countries within each type of TI on farm level were tested by running a one-way ANOVA and Scheffé's method was used for post hoc comparison using SPSS statistics 24. To this extent, non-normally distributed values were logarithmically transformed (Log10) after adding 1 unit to every observation to avoid log transformation of 0. Additionally, a Spearman's rank correlation test was run at the farm level, indicator-to-indicator, to compare different indicators. This was done for all combination of the different types of TI. The results from Bulgaria were not included in these correlations as the sampled period of the purchased data only covered one

month, making extrapolation to a period of one year unreliable. Statistical significance was set at the 5% level.

RESULTS

Antimicrobial usage expressed in treatment incidence

In relation to $TI_{DDDvetF}^{*}$, Italian and Polish farms had the highest AMU with an average $TI_{DDDvetF}^{*}$ at farm level of 36.7 and 31.8 respectively. This means for example that on average, for the 20 sampled farms in Italy, broilers were treated with antimicrobials during 37% of the rearing period. The countries with the lowest values were Bulgaria, the Netherlands and Denmark with an average $TI_{DDDvetF}^{*}$ of 3.3, 3.3 and 5.4 respectively. These three countries also represented the highest number of farms that were able to raise broilers without the use of any antimicrobials: 17 in Bulgaria, 15 in the Netherlands and 17 in Denmark. However, not all of the farms in these countries scored this well. The $TI_{DDDvetF}^{*}$ of Bulgaria, the Netherlands and Denmark, differed significantly from the other countries except for Germany. The variation between farms within the same country and between farms of different countries is presented in Table 1.

Antimicrobial usage was quantified repeatedly, each time using a different indicator. Table 2 gives an overview of the correlations between all the indicators used in this study. Both within the treatment dataset and the purchased dataset, $TI_{DDDvetF}^{*}$ and $TI_{DCDvetF}^{*}$ were highly correlated (0.99). $TI_{UDDvetF}^{*}$ showed a weaker but still strong correlation with $TI_{DDDvetF}^{*}$ (0.74) and $TI_{DCDvetF}^{*}$ (0.76). With a 0.44 correlation between $TI_{DDDvetF}^{*}$ and $TI_{DDDvetF}^{**}$, the results between the 2 datasets do not correlate highly although the same indicator for quantification was compared. The weakest correlations were seen between $TI_{UDDvetF}^{*}$ and the indicators of the purchased dataset. All results further mentioned below are based on the results from TI_{DDDvet}^{*}.

Antimicrobial usage divided over the different antimicrobial classes

Polymyxins, which were all treatments with colistin, were the most commonly used class of antimicrobials with 26% of the total AMU in the 161 participating farms (Table 3). It was the most commonly administered antimicrobial class in Spain and Italy with 43% and 50% respectively. Nevertheless, this class was only used in 4 out of 8 participating countries. Polymyxins were followed by aminopenicillins with 26% and fluoroquinolones with 16% of the total AMU of all participating countries. The latter two antimicrobial classes were used in all countries, except for Denmark. In contrast to the other countries, tetracyclines were the largest group of administered antimicrobials in Denmark, with 72% of the total usage measured in the three Danish farms using antimicrobials in the sampled batch. The only other used antimicrobial class in this country was the combination of lincomycin and spectinomycin, which was the most commonly used antimicrobial in Germany. However, lincomycin – spectinomycin was not an important class for the other countries in this study with only 5% of the total AMU.

Table 1. Antimicrobial usage (AMU) at the farm level expressed as $TI_{DDDvetF}$*/**, $TI_{DCDvetF}$*/**, and $TI_{UDDvetF}$*.

Country	NFNUA	$TI_{DDDvetF}$* avg - med min-max	$TI_{DCDvetF}$* avg - med min-max	$TI_{UDDvetF}$* avg - med min-max	$TI_{DDDvetF}$** avg - med min-max	$TI_{DCDvetF}$** avg - med min-max
Belgium	2	15.2^{b} – 11.1 0 – 59.7	$3.6^{c,d}$ – 2.7 0 – 14.6	13.2^{c} – 7.9 0 – 31.6	$12.5^{b,c,d}$ – 124.8 1.9 – 22.9	$2.7^{b,c,d}$ – 26.8 0.4 – 5.1
Bulgaria	17	3.3^{a} – 0 0 – 29.7	0.7^{a} – 0 0 – 6.4	2.1^{a} – 0 0 – 22.0	$106.1^{b,c,d}$ – 85.0 0 – 771.2	$14.2^{b,c,d}$ – 20.7 0 – 97.7
Denmark	17	5.4^{a} – 0 0 – 46.3	1.3^{a} – 0 0 – 11.4	1.7^{a} – 0 0 – 13.9	1.2^{a} – 0 0 – 11.1	0.3^{a} – 0 0 – 2.7
Germany	7	$7.7^{a,b}$ – 7.0 0 – 276.7	$1.6^{a,b,c}$ – 1.4 0 – 5.6	$7.7^{b,c}$ – 8.1 0 – 27.0	$32.7^{b,c,d}$ – 154.5 1.1 – 159.8	$6.4^{c,d}$ – 32.0 0.2 – 30.3
Italy	1	36.7^{b} – 25.4 0 – 174.5	$7.4^{c,d}$ – 5.2 0 – 24.3	17.0^{c} – 17.4 0 – 30.6	60.4^{d} – 503.2 12.2 – 166.2	12.0^{d} – 98.4 2.5 – 33.4
Poland	1	31.8^{b} – 28.4 0 – 101.0	7.2^{d} – 6.5 0 – 22.1	24.4^{c} – 21.8 0 – 40.8	$35.4^{c,d}$ – 178.0 2.9 – 209.1	$7.9^{c,d}$ – 39.4 0.7 – 45.6
Spain	4	16.3^{b} – 11.0 0 – 46.1	$3.6^{c,d}$ – 2.8 0 – 9.2	11.0^{c} – 13.6 0 – 22.7	$14.3^{b,c}$ – 108.3 0 – 61.5	$3.3^{b,c}$ – 24.5 0 – 13.3
The Netherlands	15	3.3^{a} – 0 0 – 26.5	$0.8^{a,b}$ – 0 0 – 6.1	$3.0^{a,b}$ – 0 0 – 20.4	$6.56^{b,c}$ – 40.0 0.5 – 44.5	$1.5^{a,b,c}$ – 8.7 0.1 – 10.2
Avg - med		15.0 – 7.9	3.2 – 1.8	10.0 – 8.1	335.1 – 10.2	60.8 – 2.3
Total	64					

NFNUA = Number of Farms Not Using Antimicrobials during the sampled round, avg = average, min = minimum, max = maximum, TI = treatment incidence, DDDvet = defined daily dose, DCDvet = defined course dose, UDDvet = used daily dose. $TI_{DDDvetF}$ = sum of all TI_{DDDvet} within a farm

^{a,b,c,d}Different superscripts within a column indicate significant differences

*Based on the group treatment dataset

**Based on the purchased dataset

Table 2. Correlations (Spearman's rank correlation) between the different indicators for quantifying antimicrobial usage (AMU) at farm level.

	$TI_{DDDvetF}$*	$TI_{DCDvetF}$*	$TI_{UDDvetF}$*	$TI_{DDDvetF}$**	$TI_{DCDvetF}$**
$TI_{DDDvetF}$*	1				
$TI_{DCDvetF}$*	0.99	1			
$TI_{UDDvetF}$*	0.74	0.76	1		
$TI_{DDDvetF}$**	0.44	0.43	0.36	1	
$TI_{DCDvetF}$**	0.44	0.44	0.38	0.99	1

*Based on the treatment dataset
**Based on the purchased dataset

Table 3. Percentage of the total amount of antimicrobials used (%) by country and in total.

Antimicrobial Class	NL	DE	ES	BE	IT	PL	DK	BG	Total
Aminoglycosides	-	-	6	-	-	5	-	-	2
Aminopenicillins	21	23	18	31	30	27	-	45	26
Amphenicols	-	-	-	-	-	2	-	-	<1
Fluoroquinolones	12	7	19	15	6	31	-	31	16
Linco&Spec	-	28	-	14	-	1	28	-	5
Lincosamides	-	-	-	-	-	-	-	23	1
Macrolides	-	-	7	<1	6	1	-	-	3
Other quinolones	-	-	-	3	-	-	-	-	<1
Penicillins	-	-	-	-	-	3	-	-	1
Polymyxins	-	20	43	-	50	14	-	-	26
Tetracyclines	19	21	7	16	-	15	72	-	12
Trim&Sulfa	48	-	-	21	9	2	-	-	7

Linco&Spec=Lincosamides and spectinomycin, Trim&Sulfa=Trimethoprim and sulphonamides, BE=Belgium, BG=Bulgaria, DE=Germany, DK=Denmark, ES=Spain, IT=Italy, NL=The Netherlands, PL=Poland

On the first day of production, about 1 out of 4 farms (24%) started an antimicrobial treatment, followed by 11% and 5% on day 2 and 3 respectively (Fig. 1). As a result, 34% of farms were treating their broilers with antimicrobials on day 3. Afterwards, the number of farms treating gradually declined to 2% on day 13, but increased again to fluctuate around 10% during the 3rd and 4th week. When looking at the age at treatment in the different countries, the early peak showed up everywhere with a maximum of treating herds on day 3 of 60% in Poland, whereas this was only 10% in Denmark. More remarkably for the Danish farms, was the fact that they administered all their antimicrobials within the first 3 days of production (data on country level not shown).

Antimicrobial usage over the period of one production cycle in broilers

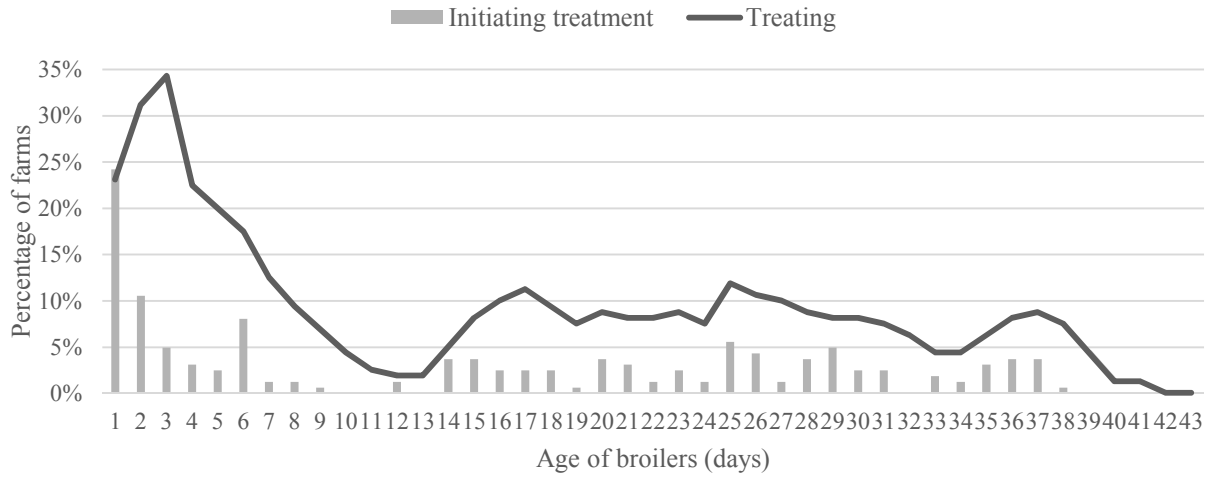

Fig. 1 Age of broilers in relation to antimicrobial usage.

DISCUSSION

Study design

Some studies have already described AMU for different species together (Bos et al., 2013; Filippitzi et al., 2006), others for only one species in particular (Persoons, 2010; Timmerman et al., 2006) In most cases they only covered one country or did not use the same methodology over countries (Bos et al., 2013; Filippitzi et al., 2014; Persoons, 2010; Timmerman et al., 2006). When multi-country studies on AMU did use the same methodology, results were mostly based on sales data making detailed description of AMU at farm level or direct comparison per species challenging (Bengtsson & Wierup, 2006; EMA, 2015a; Grave et al., 2006). To the authors' knowledge this is the first multi-country study that was able to report on AMU in broilers in such detail. The same sampling protocols were used within the 8 participating countries. After data collection the same methodology was used to quantify AMU, allowing comparison of the results at the farm level within the same country but also between different countries.

Treatment incidence

DDDvet values have been determined by ESVAC based on the recommended dosage found in the summary of product characteristics (SPC) of the drugs in 9 European countries (EMA, 2015b). However, ESVAC reports these values as technical units of measurement that should not be considered to reflect the recommended daily dose in all circumstances as they are often a compromise between different advised doses for the same active compound in different countries or in different commercial products in the same country (EMA, 2016; Postma et al., 2015). In contrast, the used daily dose (UDD) can differ between and within herds, as it represents the administered dose of a drug on the farm itself. The flexibility of UDD makes the indicator robust to noncompliance with the SPC and therefore it better represents the true exposure at the farm level (Chauvin et al., 2004; Timmerman et al., 2006). Yet the limitation of the UDD is the requirement for very detailed herd level data collection. Nonetheless, from the obtained results it appears that $TI_{DDDvetF}^{*}$ and $TI_{UDDvetF}^{*}$ seem to correlate strongly (0.74).

In general, differences between UDDvet and DDDvet are caused by erroneous estimations concerning the age and weight of the animals at the moment of treatment, adaptation of the dosage towards the package size, treatment for an indication different from the main indication or deviations from the main therapeutic protocol (Timmerman et al., 2006). However, on a meta level the results did correlate quite well. This is good news as it indicates that the DDDvet, which is more convenient to report on AMU in different countries in a harmonised manner, does relatively accurately describe the actual exposure as measured by means of the UDDvet. Next to DDDvet and UDDvet, TI was also calculated using DCDvet to describe the number of courses rather than the number of treatment days. Defined course dose is one of the parameters used in the ANSES report on AMU (ANSES, 2014) and is mentioned in the reflection paper of the European Medicines Agency (EMA) on the collection of AMU data. Defined course dose gives more information about the average duration of treatment when compared with the DDDvet, as it represents the mean dosage of a full treatment course instead of just 1 day of treatment (EMA, 2013). However, results of both TI_{DDDvet}^{*} and TI_{DCDvet}^{*} were very strongly correlated (0.99). So both give similar results, only the interpretation is different. The same correlations were found within the purchased dataset. Nevertheless, when comparing the same indicator between datasets (e.g. TI_{DDDvet}^{*} and TI_{DDDvet}^{**}), low correlations were found. This can be explained by the differences between the two datasets. The group treatment dataset only covers one production round while the purchased dataset covers one year. It should also be mentioned that not all purchased products are necessarily used, which could lead to an overestimation of AMU when quantification is based on the purchased data (Collineau et al., 2017).

Amount of antimicrobials used

This study has shown that the use of antimicrobials differs greatly between farms, both within and between countries. The same finding was reported concerning AMU by a study conducted in pigs in Belgium, France, Germany and Sweden (Sjölund et al., 2016). In addition, other studies conducted at the farm level also reported a large between-farm variation (Bos et al., 2013; Callens et al., 2012; Persoons et al., 2012; Timmerman et al., 2006). When looking at the average $TI_{DDDvetF}^{*}$ between countries, the Italian and Polish farms had the highest average antimicrobial consumption per farm while the lowest were measured among the Bulgarian, Dutch and Danish farms. This large variation is also seen in the $TI_{DDDvetF}^{*}$ between farms within the same country.

Antimicrobial classes used

There was a noticeable focus on the use of certain antimicrobial classes, with 3 classes being responsible for almost 70% of all the antimicrobials administered in this study. Furthermore, each country had a different distribution in antimicrobial classes used. This variation in usage of antimicrobial classes between countries was also found for pigs (Sjölund et al., 2016) and in general for AMU in veterinary medicine as reported by the ESVAC reports (European Medicines Agency, 2017). These variations may be the result of a multitude of drivers. First of all different levels of AMR between countries can possibly explain these findings (EFSA, 2017). Also differences in availability of registered antimicrobial products between countries and the presence or absence of a specific legislation to reduce the use of critically important antimicrobials (CIA) may be of importance (Ferreira & Staerk, 2017; Speksnijder et al., 2015). Besides these, other explanations have been suggested in the literature, such as economic incentives or the experience of the veterinarian (Gibbons et al., 2013). By publishing a list with CIA, the WHO has tried to steer veterinarians away from the use of those antimicrobials, pivotal for human healthcare. Nevertheless, fluoroquinolones and polymyxins belonged to the

most commonly used classes in this study while being on this list of CIA with highest priority (WHO, 2009). However it should be noted that polymyxins were only recently added to the list in 2016, while sampling already started in May 2014.

Age of treatment

When looking at the age at treatment, certain times during the rearing period stand out because of their high antimicrobial usage. The first and biggest peak in the first 3 days of production represented 32% of the total AMU. This peak in AMU might be caused by the routinely prophylactic use of antimicrobials that are applied to prevent disease. This type of mass-medication is seen as a cheap and easy solution for disease prevention. A study in pigs showed that 93% of all group treatments can be categorized as prophylactic AMU (Callens et al., 2012). More recently, in 2015, the European Commission published a report with guidelines for the prudent use of antimicrobials in veterinary medicine. In this report they call for action regarding the prophylactic and recurrent group medication of poultry, which is often done right before or after transport of day-old chicks or is used to tackle losses in productivity (EU Commission Notice, 2015).

ACKNOWLEDGEMENTS

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ANTIMICROBIAL TREATMENT DATA FROM FARROW-TO-FINISH PIG FARMS SHOWING OPPORTUNITIES FOR IMPROVING RESPONSIBLE ANTIMICROBIAL USE IN EUROPE

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SUMMARY

To quantify antimicrobial usage (AMU) in pigs on farm level data were collected at 160 farrow-to-finish pig farms in eight European countries. The majority of antimicrobial treatments were applied in the early phase of production, with peaks during week 1, 4 and 9 of the rearing period. Pigs from birth to slaughter were treated with antimicrobials in half of the farms during at least 8.8% of their rearing period, with a large variation within and between countries. Colistin, a critically important antimicrobial for human medicine, represented 22.7% of the total use and was especially used in weaned piglets for intestinal disorders. Higher AMU in young pigs was associated with higher use in older pigs, which suggests that a good start for piglets is of utmost importance for responsible use of antimicrobials throughout the production cycle. Furthermore, this study showed that it is feasible to rear pigs without systematic use of antimicrobials, since such farms were included.

INTRODUCTION

During the World Health Assembly of 2015, the World Health Organization (WHO) recognised the importance of the public health problem posed by antimicrobial resistance (AMR) (WHO, 2017a). Given the fact that there are strong indications of animal–human transmission of AMR (da Costa et al., 2013; Evers et al., 2017) and that antimicrobial usage (AMU) is the strongest driver for the selection of AMR (Chantziaras et al., 2014; European Centre for Disease Prevention and Control (ECDC, 2015)), it is of utmost importance to reduce veterinary AMU towards a responsible use to retain a multitude of treatment options (Sjölund et al., 2016).

To reduce the unnecessary use of antimicrobials in food-producing animals, the WHO and the World Organisation For Animal Health (OIE) recommend that the quantities of antimicrobials used in food-producing animals for disease prevention and treatment are monitored (OIE, 2016; WHO, 2017a). In Europe sales data of antimicrobials is monitored in a standardised manner by the European Surveillance of Veterinary Antimicrobial Consumption

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(ESVAC) project and reports show that considerable differences exist between countries (ESVAC, 2017). However, in the ESVAC reports no distinction is made between species, whereas it is known that there are differences in AMU between species and the pork industry is responsible for a large share of AMU in animal production (DANMAP, 2013; Filipitzi et al., 2014; MARAN, 2017).

To quantify AMU various indicators are available and results can differ substantially depending on the method used (Collineau et al., 2017). The sales figures at country level of ESVAC are only a crude measurement of actual consumption and do not take into account the large differences in molecular weight between different antimicrobial compounds nor the demographic composition of the animal population (Sjölund et al., 2016). To reduce AMU towards a more responsible use at farm level, AMU quantification should allow for benchmarking between farms and studying the associations between animal health, production characteristics and AMR. Therefore more detailed information such as the assessment of exposure (e.g. administration dose and route) is required (Collineau et al., 2017).

To be able to better define what a more responsible use of antimicrobials embraces this study aimed to quantify AMU for pigs at the farm level in nine European countries in a standardized manner. This study was conducted within the European research project EFFORT (Ecology from Farm to Fork Of microbial drug Resistance and Transmission, www.effort-against-amr.eu). This project investigates the epidemiology and ecology of AMR in food-producing animals, the environment and humans to quantify AMR exposure pathways for humans.

MATERIALS AND METHODS

Selection of herds

A cross-sectional study was conducted in eight countries: Bulgaria, Belgium, Denmark, Germany, Italy, Poland, Spain and the Netherlands. In each participating country, 20 conventional integrated pig farrow- to- finisher non-mixed farms were selected as previously described by Munk et al. (2017). The farms needed to have a minimum of 150 sows and 600 fatteners and were preferably randomly selected from a list of eligible farms, using regional stratification whenever possible. Deviations from this protocol are described in the supplementary material of Munk et al. (2017). Written informed consent from the participating farmers was obtained.

Collection of antimicrobial consumption records

The farms were visited by researchers within the EFFORT project between May 2014 and December 2015. During the herd visits, detailed information was collected about the administration of antimicrobials as a group treatment to the sampled batch of fattening pigs during their entire rearing period. A group treatment was defined as each treatment applied at the same moment to all animals present in, at least, the smallest housing unit. Individual treatments were not taken into account for further analysis. Information on indications for treatment (general, intestinal, locomotive, respiratory, urogenital, nervous or other disorders) was also collected. Data collection was based on a questionnaire developed within the EFFORT consortium and can be provided upon request.

Data processing

Data were entered by the researchers, having visited the farms, into EpiData version 3.1 software (EpiData Association, Denmark). A first data quality control was performed (e.g. missing values, matching farm identifications) and files were corrected if necessary. EpiData files were converted to Microsoft Excel and a second, more in-depth data quality control was performed (e.g. units, outliers).

Quantification of antimicrobial consumption

Based on Defined Daily Dose for animals (the assumed average maintenance dose per day per kg body weight for the main indication in a specified species; DDDvet) and long-acting (LA) factor (a value to represent the duration of activity of a long-acting product) the treatment incidence (TI) indicator was used to quantify the antimicrobial consumption, in accordance with Timmerman et al. (2006) as seen in Eq. (1).

$$\text{TI} = \frac{\text{Total amount of antimicrobials administered}}{\text{DDDvet x number of days at risk x kg animal at risk}} \text{ x LA factor x 100 pigs at risk} \qquad (1)$$

The TI expresses the percentage of pigs receiving a dose of antimicrobials each day, or equivalently, during the percentage of its life a pig is treated with antimicrobials (Timmerman et al., 2006).

DDDvet values were used as provided by ESVAC (EMA, 2016). Whenever for a given combination of active substance and administration route no DDDvet values were defined by ESVAC, the values as described by Postma et al. (2015) were used or the information was obtained from the summary of product characteristics (SPC). According to ESVAC, the same DDDvet values are assigned to active substances, regardless of if they are in a single substance product or a combination product. However, when the dose of at least one of the active substances differed substantially between single substance products and combination products, as for combination products of lincomycin and spectinomycin and combination products of trimethoprim and a sulphonamide, more than one DDDvet value per administration route for those active substances was provided. LA factors were used as described by Postma et al. (2015).

TI calculations were performed for each administered antimicrobial product per age category. In accordance with Sjölund et al. (2016), the TIs of suckling piglets, weaned piglets and fatteners were combined and recalculated into a standardised lifespan of 200 days to correct for possible differences in ages at slaughter between herds (TI200), based on the number of days for the actual rearing period of a herd.

To obtain an impression of the treatment duration for LA formulations the number of days the treatment was applied was multiplied with the LA factor.

Statistical analysis

Differences in TIs between countries were tested by means of the Kruskal-Wallis rank sum test. A non-parametric test was used, since TIs at country level deviated considerably from normality, even after logarithmic transformation. To take into account the multiple comparisons the Benjamini and Hochberg (1995) method was used for post hoc comparison. The relation between TIs for the different age categories were compared by fitting Loess curves after the TI variables were log transformed to deal with the problem of a skewed distribution.

Before transforming the data a value of 1 was added to all observations to overcome the problem of the observations where the TI for a particular age category was 0 (Sjölund et al., 2016). Furthermore, cross-tabulation was used and graphs were prepared to visualise the results. For quantification of AMU and statistical analysis R version 3.3.1 software (https://cran.r-project.org) was used.

RESULTS

A total of 781 antimicrobial treatments was applied by 159 farms, meaning that 21 farms (11.7%) did not apply antimicrobial group treatments during the rearing period of the sampled batch. The majority of the treatments were applied in the early phase of production, with peaks during week 1 (16.9%), 4 (10.9%) and 9 (7.2%) of the rearing period based on 751 treatments (the time of treatment was missing for 30 observations) (Fig. 1).

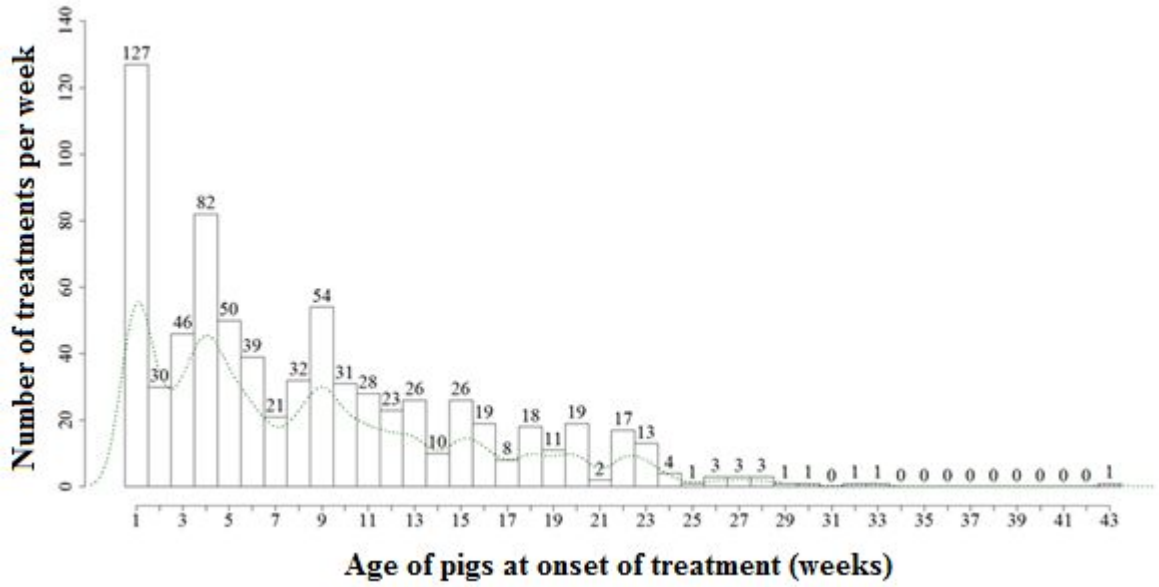

Fig. 6 This histogram shows the number of antimicrobial treatments that were started per week based on 751 treatments (30 times of treatments missing). Three peaks of age at treatment onset appear, namely during week 1, 4 and 9.

The median TI200 varied considerably between farms and countries (Table 1), with a median TI200 of 8.8 and a maximum of 129.1. This means that in half of the farms growing pigs received a daily dose of antimicrobials during at least 8.8% of their rearing period. The lowest median TI200 were observed in Dutch and Bulgarian herds, while the highest median TI200 was observed in Spanish herds. The highest AMU was observed during the early phase of life: in terms of TI, 35% of all treatments were performed during the suckling phase (TI sucklers: min 0.0; median 7.0; max 264.6) and 56% during the nursery period (TI weaners: min 0.0; median 13.5; max 398.7). During the finishing period 9% of the treatments were administered (TI fatteners: min 0.0; median 0.3; max 151.6). Positive associations between age categories were observed (sucklers, weaners and fatteners) (Fig. 2).

The majority of the antimicrobials were administered through feed or water (70%), 30% was administered parentally. Only 0.006% of the TI consisted of topical treatments. Denmark administered most of the antimicrobials parenterally (75%), with other countries administering variable levels via this route (Poland 59%, Italy 34%, Germany 32%, Belgium 29%, Bulgaria

25%, the Netherlands 19% and Spain 12%). The mean treatment duration for parenterally administered antimicrobials was 2.6 days [min 1; median 3; max 7] for non-LA formulations and 5.2 days [1.8; 5; 28] for LA formulations. For oral treatments this was 10.6 days on average [1; 7; 80] and 2.2 days [1; 1; 5] for topical treatments. Some treatments (5.4%) were applied for a period of at least 21 consecutive days; all of them were oral treatments.

In terms of TI, aminopenicillins (29.5%), polymyxins (22.7%) were the most frequently used antimicrobial classes, followed by macrolides (13.0%) and tetracyclines (10.5%) (Table 2). Aminopenicillins, polymyxins and tetracyclines were mainly used for weaners (61.8%, 81.7% and 74.7% of the use within the respective class), while cephalosporins and macrolides were more frequently applied for suckling piglets (93.0% and 54.0% of the use within the respective class). Nearly all treatments with cephalosporins, which all belonged to the third and fourth generation, were long acting formulations (99.7%). Other antimicrobial classes where LA formulations were used, were penicillins (82.8%), macrolides (60.5%), aminopenicillins (17.9%), amphenicols (14.8%), tetracyclines (1.2%) and fluoroquinolones (0.9%). The most frequent indications for treatment were general (34.1%), intestinal (25.0%) and respiratory disorders (18.7%), based on 769 treatment indications (for 12 observations the indication for treatment was missing). The use of antimicrobial classes varied by treatment indication: e.g. for intestinal disorders polymyxins were most frequently administered (41.8%) and for respiratory disorders these were aminopenicillins (30.8%) and macrolides (24.7%).

DISCUSSION

By using a standardised and detailed methodology to quantify AMU at the farm level this study gained more insight into the quantitative and qualitative use of antimicrobials regarding time of treatment, antimicrobial substances and administration route in 160 farrow-to-finish farms in eight European countries. With sampling only 20 farms per country, the main goal was not an exact description of AMU at country level, but rather a more detailed description at farm level. Although the approach was comparable to the study of Sjölund and co-workers (2016), which described AMU in four European countries, the present study covered eight European countries and obtained additional results, such as the time and duration of treatment. Moreover, the ESVAC defined DDDvet values were used, which were based on detailed dosing data from nine European Union Member States.

In accordance with other studies (Callens et al., 2012; Sjölund et al., 2016), the highest AMU was observed in weaners (56% of total TI), followed by sucklers (35% of total TI) for all countries except Denmark, where AMU was on average the highest in sucklers (see below). More specifically, the majority of treatments was applied during week 1 (16.9%), 4 (10.9%) and 9 (7.2%) of the rearing period. As already argued by Sjölund and co-workers (2016), these results strongly suggest that it is common practice in many pig herds to apply treatments to entire batches of pigs at strategic time points when pigs are judged most likely to contract disease, i.e. at birth and castration (week 1), at weaning (week 4) and at the start of the finishing period (week 9). Moreover, in accordance with Sjölund and co-workers (2016), positive associations in AMU between the age categories (sucklers-weaners, sucklers-fatteners and weaners-fatteners) were observed, indicating that higher antimicrobial usage at a young age also associated with higher use at an older age. Possible reasons for these associations are the attitude of the farmer (Visschers et al., 2015), the overall higher disease pressure in some herds (Sjölund et al., 2016) and the higher disease susceptibility following the effect of AMU at young age (Callens et al., 2014). Therefore, a crucial step towards more responsible AMU should be the reduction of prophylactic and metaphylactic AMU at young age, since this seems

Table 1. Overview of the group treatments of the sampled batch expressed in treatment incidences (TI) based on Defined Daily Dose animal (DDDvet) values. Mean values are shown with the 5th and 95th percentile between square brackets and minimum, median and maximum values between round brackets.

Country	Number of treating farms	Number of treatments per farm	TI200	TI sucklers	TI weaners	TI fatteners
Belgium	20	3.4 (1; 3; 10)	$20.0^{c,d}$ [0.8; 57.0] (0.3; 9.9; 129.1)	$30.4^{a,c}$ [0.0; 129.9] (0.0; 16.6; 134.4)	$34.6^{c,d,e}$ [0.0; 136.0] (0.0; 18.9; 147.7)	$11.7^{b,c}$ [0.0; 37.6] (0.0; 0.2; 151.6)
Bulgaria	18	1.2 (0; 1; 3)	1.1^{e} [0.0; 2.8] (0.0; 0.5; 6.7)	0.6^{b} [0.0; 2.8] (0.0; 0.0; 8.7)	2.2^{f} [0.0; 8.0] (0.0; 0.0; 21.0)	$0.4^{c,d}$ [0.0; 2.2] (0.0; 0.02; 3.7)
Denmark	19	5.9 (0; 6; 10)	$13.8^{c,d}$ [0.3; 28.0] (0.0; 10.1; 52.5)	28.8^{a} [0.0; 83.7] (0.0; 19.6; 95.2)	$14.7^{d,e}$ [0.0; 66.7] (0.0 ; 7.2; 71.9)	$1.7^{c,d}$ [0.0; 5.9] (0.0; 0.2; 7.2)
Germany	19	2.9 (0; 2; 7)	7.3^{d} [0.4; 17.4] (0.0; 6.0; 39.9)	$10.5^{c,d}$ [0.0; 41.9] (0.0; 3.0; 79.9)	13.9^{e} [0.0; 55.1] (0.0; 3.8; 90.2)	$3.6^{b,c}$ [0.0; 18.7] (0.0; 0.2; 23.4)
Italy	20	8.7 (2; 10; 11)	23.8^{b} [8.1; 50.9] (5.4; 18.1; 74.1)	51.4^{a} [0.0; 126.5] (0.0; 44.6; 126.5)	86.1^{b} [16.4; 207.6] (8.2; 49.3; 369.1)	9.7^{b} [0.0; 27.7] (0.0; 5.1; 52.7)
Poland	20	4.7 (2; 4; 10)	$14.9^{b,c}$ [3.7; 27.4] (1.1; 13.7; 37.7)	48.3^{a} [0.0; 210.9] (0.0; 19.8; 219.3)	$38.7^{b,c}$ [3.7; 88.1] (0.3; 41.2; 89.4)	3.5^{b} [0.0; 8.2] (0.0; 3.3; 8.2)
Spain	20	9.9 (5; 10; 17)	59.4^{a} [14.8; 109.1] (8.0; 60.0; 111.6)	64.1^{a} [0.0; 117.4] (0.0; 70.6; 264.6)	150.7^{a} [0.0; 285.1] (0.0; 158.1; 398.7)	27.6^{a} [0.7; 57.5] (0.3; 31.1; 61.7)
The Netherlands	6	0.5 (0; 0; 3)	1.9^{f} [0.0; 5.1] (0.0; 0.0; 23.4)	2.3^{b} [0.0; 7.9] (0.0; 0.0; 40.5)	9.6^{f} [0.0; 16.8] (0.0; 0.0; 165.2)	0.1^{e} [0.0; 0.1] (0.0; 0.0; 2.4)

Different superscripts denote significant differences ($p < 0.05$) between countries based on a Kruskal-Wallis test and pairwise comparisons between countries with corrections for multiple testing using Benjamini and Hochberg method (Benjamini and Hochberg, 1995)

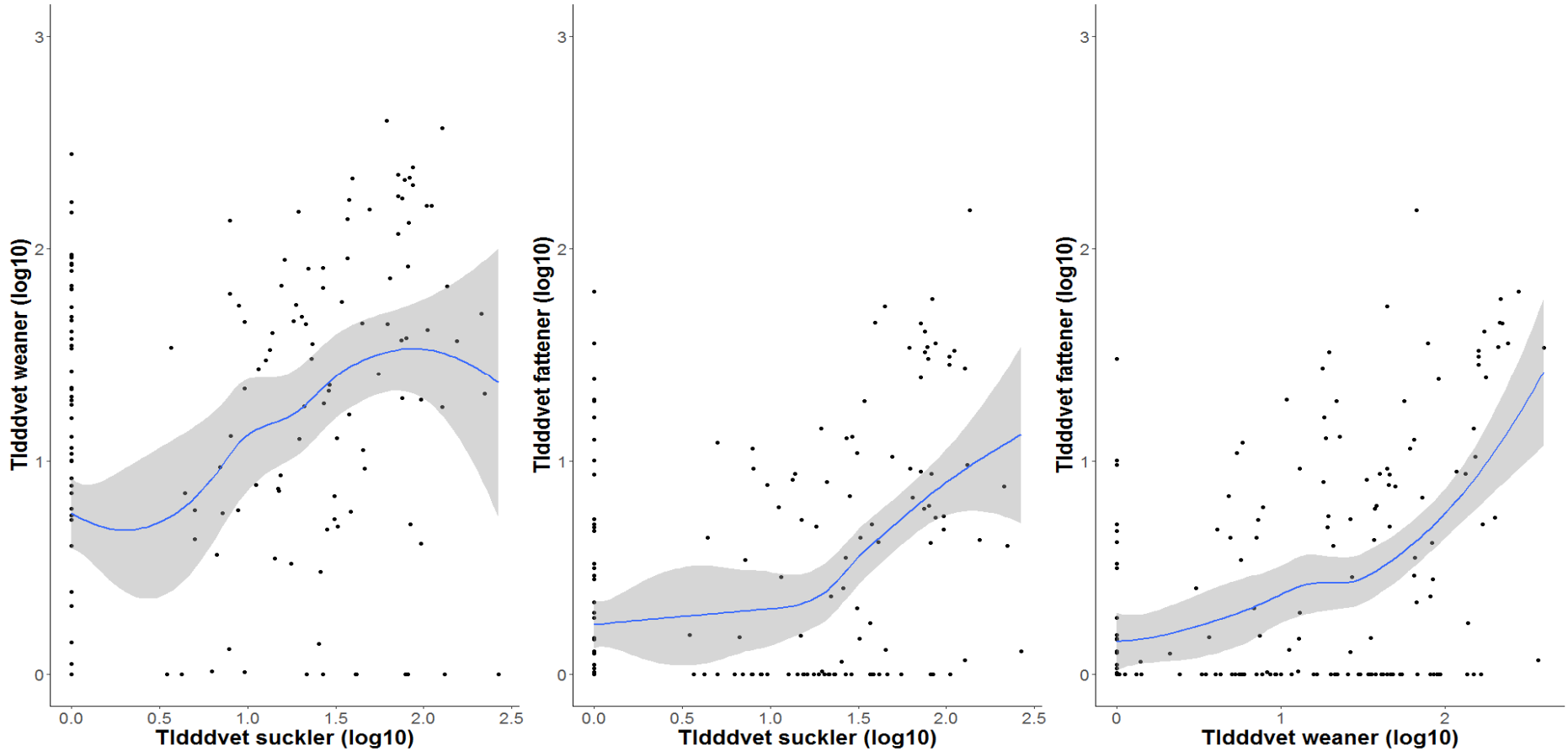

Fig. 2 After log transforming the data, Loess curves (with 95% confidence band) were fitted to compare antimicrobial usage between age categories (sucklers, weaners and fatteners). Antimicrobial usage is expressed in treatment incidences (TI) based on Defined Daily Dose animal (DDDvet) values.

Table 2. Proportion of the amount of antimicrobials used (%) by antimicrobial class in relation to the total use and by country.

Class	Total	BE	BG	DE	DK	ES	IT	NL	PL
Aminoglycosides	1.7	-	13.8	4.5	4.2	1.0	0.5	-	5.3
Aminopenicillins	29.5	45.6	11.0	46.2	33.8	24.8	26.7	58.4	24.0
Amphenicols	0.6	-	12.6	-	-	0.1	2.5	-	0.04
Cephalosporins	6.8	9.4	4.2	4.3	-	2.4	14.7	-	11.8
Fluoroquinolones	1.3	-	12.1	3.4	-	0.7	2.8	-	1.7
Linco&Spec	0.5	1.3	-	-	1.1	-	0.003	-	0.003
Lincosamides	1.1	-	7.1	1.5	0.3	0.6	3.7	-	-
Macrolides	13.0	6.8	1.3	17.5	26.5	12.6	13.4	-	10.9
Penicillins	5.2	2.2	-	2.2	13.4	0.8	0.01	-	27.8
Pleuromutulins	3.4	-	0.6	-	3.2	7.5	1.2	-	1.8
Polymyxins	22.7	7.5	36.1	11.4	0.5	33.0	27.0	29.1	7.6
Sulfonamides	0.3	-	-	-	-	-	1.5	-	-
Tetracyclines	10.5	6.3	1.2	9.0	14.3	16.0	4.9	3.1	9.0
Trim&Sulfa	3.2	20.8	-	-	2.6	0.5	0.9	9.4	0.1

Linco&Spec=Lincosamides and spectinomycin; Trim&Sulfa=Trimethoprim and sulphonamides; BE=Belgium, BG=Bulgaria, DE=Germany, DK=Denmark, ES=Spain, IT=Italy, NL=The Netherlands, PL= Poland

to have a major impact on the entire rearing period (WHO, 2017a). In a recent study, the restriction of prophylactic use in weaner pigs by removing antimicrobials from the feed had minimal effects on health and welfare indicators (Diana et al., 2017).

The fact that no group treatments were applied in 11.7% of the farms (mainly Dutch farms) for the sampled batch, showed that it is possible to rear pigs without systematic use of antimicrobials. Furthermore, in the other countries there was at least one farm that did not use antimicrobials in either suckling piglets, weaners or fatteners during the observed rearing period. Since individual treatments were not taken into account in the present study, it is still possible that some of the sampled animals were treated individually. Nonetheless, this should not pose a problem, since responsible use of antimicrobials does not involve the ban of these essential tools for ensuring the health of people and animals, but rather a targeted use when indicated and strictly necessary.

Denmark was the only country where a higher AMU was observed in suckling piglets compared to weaners. Together with Poland it was also the only country where the majority of the treatments were applied parenterally (75% and 59% for Denmark and Poland, respectively). It is difficult to compare these results with other Danish data, since results on AMU in Danish pigs are usually reported for suckling piglets and sows together (Jensen et al., 2012; DANMAP, 2016). However, similar findings, i.e. a higher AMU in suckling piglets compared to weaners in combination with a majority of parenteral treatments, were also observed in Swedish pig farms (Sjölund et al., 2016). Since an overall low AMU was reported for the Swedish pig farms, it was argued that how antimicrobials are applied could also explain differences in AMU. However, in the present study the majority of parenteral treatments was not reflected in a low TI200 for Denmark. Nonetheless, this can partially be explained by a shorter rearing period in Danish pig farms (median duration: 158 days (Denmark) versus 191 days (other countries)), due to a short finishing period (median duration: 80 days (Denmark) versus 120 days (other

countries)). As such, the suckling phase and nursery period (where the majority of the antimicrobials are used) have a relatively large impact on the TI200. Yet, oral administration of antimicrobials is likely to be more easily applied to entire batches compared to parenteral treatments and may therefore have a larger impact on AMU. Furthermore, in the present study oral treatments were generally applied for longer periods compared to parenteral treatments. Finally, studies have shown that oral administration of antimicrobials selects more for AMR compared to parenteral treatments (Wiuff et al., 2003; Burow et al., 2014; Chantziaras et al., 2017). Therefore, another factor towards a more responsible AMU should be the consideration of parenteral treatment of individuals rather than oral treatment of an entire batch, whenever possible (WHO, 2017a).

In this study polymyxins were frequently used in weaned piglets for intestinal disorders. Polymyxins (colistin) have recently been added to the list of critically important antimicrobials for human medicine (WHO, 2017b) and the use of such antimicrobials in food-producing animals is discouraged (WHO, 2017a). Furthermore, a frequently used alternative to control post-weaning diarrhoea is the use of zinc oxide, but the Committee for Medicinal Products for Veterinary Use (CVMP) recently proposed a ban on zinc oxide because of the environmental risk (CVMP, 2016). The use of other critically important antimicrobials for human medicine such as third and fourth generation cephalosporins and fluoroquinolones was relatively low. However, in accordance with previous studies (Callens et al. 2012; Sjölund et al. 2016), third and fourth generation cephalosporins were frequently used in suckling piglets in Belgian herds. The same holds true for Italian and Polish herds. According to a recent Belgian law the use of third and fourth generation cephalosporins and fluoroquinolones is only allowed following a laboratory analysis of samples from the diseased animals in combination with an antimicrobial susceptibility test that indicates that no non-critically important antimicrobials are active (Royal Decree 21 July 2016). As a result, the use of third and fourth generation cephalosporins and fluoroquinolones in 2016 was already 53.1% lower compared to 2015 and is expected to be further reduced in the following years (BelVetSAC, 2017). This illustrates that strict regulations on the use of critically important antimicrobials for human medicine in food-producing animals can be very beneficial in achieving a more responsible AMU.

Besides a responsible AMU when antimicrobials are actually indicated, responsible AMU also embraces the prevention of animals becoming diseased. Disease prevention includes for instance vaccination and biosecurity. Studies have already shown that by guiding farmers in their farm management this preventive approach substantially reduced AMU without jeopardising animal health (Collineau et al., 2017; Postma et al., 2017). Moreover, despite the financial investment in preventive measures, farmers increased their economic profits (Rojo-Gimeno et al., 2016; Collineau et al., 2017). The results of the present study in combination with the aforementioned studies show that it is possible to rear pigs with minimum usage of antimicrobials and all stakeholders should be motivated to continue to work on the reduction of AMU.

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SOCIETY FOR VETERINARY EPIDEMIOLOGY AND PREVENTIVE MEDICINE

PAST VENUES AND ORGANISERS OF ANNUAL MEETINGS

Year	Venue	Organiser(s)
1983	Southampton	Davies & Thrusfield
1984	Edinburgh	Thrusfield
1985	Reading	Thrusfield
1986	Edinburgh	Thrusfield
1987	Solihull	Thrusfield
1988	Edinburgh	Thrusfield
1989	Exeter	Howe
1990	Belfast	McIlroy
1991	London	Jones
1992	Edinburgh	Thrusfield
1993	Exeter	Howe
1994	Belfast	Menzies
1995	Reading	Paterson
1996	Glasgow	Reid
1997	Chester	Clarkson
1998	Ennis, Ireland	Collins
1999	Bristol	Green
2000	Edinburgh	Thrusfield & Mellor
2001	Noordwijkerhout	van Klink
2002	Cambridge	Wood & Newton
2003	Warwick	Green
2004	Martigny	Stärk
2005	Nairn	Gunn
2006	Exeter	Peeler
2007	Dipoli	Virtala & Alban
2008	Liverpool	Pinchbeck & Robinson
2009	London	Verheyen & Pfeiffer
2010	Nantes	Fourichon & Hoch
2011	Leipzig	Thulke & Lange
2012	Glasgow	Parkin & Others
2013	Madrid	Martínez-López & Vargas Alvarez
2014	Dublin	More & Collins
2015	Ghent	Mintiens & Dewulf
2016	Elsinore	Nielsen & Halasa
2017	Inverness	Gunn & Reeves

PAST PRESIDENTS

1982-’83	G. Davies
1983-’84	P.R. Ellis
1984-’85	G. Gettinby
1985-’86	R.W.J. Plenderleith
1986-’87	M.V. Thrusfield
1987-’88	K.S. Howe
1988-’89	A.M. Russell
1989-’90	S.G. McIlroy
1990-’91	J.E.T. Jones
1991-’92	J.M. Booth
1992-’93	E.A. Goodall
1993-’94	R.G. Eddy
1994-’95	J.T. Done
1995-’96	G.J. Gunn
1996-’97	M.S. Richards
1997-’98	J.D. Collins
1998-’99	F.D. Menzies
1999-’00	K.L. Morgan
2000-’01	S.W.J. Reid
2001-’02	A.D. Paterson
2002-’03	L.E. Green
2003-’04	J.L.N. Wood
2004-’05	E.G.M. van Klink
2005-’06	D.J. Mellor
2006-’07	E. J. Peeler
2007-’08	J. R Newton
2008-’09	L. Alban
2009-’10	D.U. Pfeiffer
2010-’11	L.A. Kelly
2011-’12	C. Fourichon
2012-’13	T.D.H. Parkin
2013-’14	K.L.P. Verheyen
2014-’15	K. Mintiens
2015-’16	H.H. Thulke
2016-’17	A. Lindberg
2017-’18	L.R. Nielsen

PLENARY TALKS

Year	Gareth Davies Lecture	Conference Opening Plenary
2018	Klaus Depner African swine fever: Lessons learned about the epidemiology, politics and practical implementation of control measures	Päivi Rajala-Schultz Veterinary epidemiology at the intersection of livestock disease, production and animal welfare
2017	Theresa Bernado TRENDS: Technology, Research, Epidemiology, Networks, Data & Surveillance	Tine Hald Source attribution: Translating science into public health action
2016	Bernhard Url The foundation of science-based risk assessment for decision support on food safety and animal health in EU	Mirjam Nielen Evidence-based veterinary medicine needs clinical epidemiology
2015	Piet Vanthemsche Preventive Veterinary Medicine as an essential part of sustainable animal production	Crawford Revie Hype and Hysteria: Should veterinary epidemiologists really care about Big Data?
2014	Ian Gardner Bridging the gap in infectious disease epidemiology between aquatic and terrestrial food animals: challenges and future opportunities	Nils Toft Confessions of a wannabe Bayesian
2013	Andreas Hensel Dioxins, EHEC and strawberries: Risk assessment and risk communication in practice	José Manuel Sánchez-Vizcaíno The Spanish experience on the control and eradication of infectious diseases: from the old to the current system
2012	Stuart Reid Evidence-based prevention: well done or rare	Didier Boichard Genomic selection: an opportunity for improving health of farm animals
2011	Karin Schwabenbauer From science to policy - the case of classical swine fever (CSF) control	Dominic Mellor The trouble with epidemiology: the tyranny of numbers
2010	David Waltner-Toews Beyond one world, one health and ecohealth…what's out there?	James Wood From pathogen adaption to host ecology: epidemiological and experimental contributions to the understanding of emerging infectious diseases
2009	Jørgen Westergaard The interaction between veterinary science, legislation and management in animal disease control in the European Union	Katharina Stärk Food safety challenges in a global market – are we ready?

2008	Paul Fine Infectious disease eradication – meanings and implications	Kenton Morgan For the benefit of Mr Kite
2007	Yrjö Gröhn Food supply veterinary medicine: Modelling of production, health and food safety	Laura Green Improving Animal Health
2006	David Galligan From partial budgets to real options - concepts in animal health economics	Nigel French Understanding human exposure to zoonoses from food and the environment: The application of molecular tools and modeling
2005	Bill Reilly From TB to VTEC: The changing epidemiology of foodborne zoonoses	Simon More Towards eradication of bovine tuberculosis in Ireland: A critical review of progress
2004	Ulrich Kihm BSE and the stable to table concept	Gary Smith Spatial models of infectious disease in the USA: a crisis of conference and confidentiality
2003	Sir David Cox The current state of statistical science	Ynte Schukken Molecular and mathematical epidemiology of bovine mastitis
2002	George Gettinby Informatics and epidemiology – the first 400 years	Bryan Grenfell Deterministic and stochastic influences on the dynamics and control of infectious diseases
2001	Will Houston Science politics and animal health policy: epidemiology in action	Mart de Jong Design and analysis of transmission experiments
2000	Jim Scudamore Surveillance – past, present and future	Dirk Pfeiffer Spatial analysis – a new challenge for veterinary epidemiologists
1999	Aalt Dijkhuizen The 1997/98 outbreak of classical swine fever in the Netherlands: lessons learned from an economic perspective	Mark Woolhouse Understanding the epidemiology of scrapie
1998	Wayne Martin Art, science and mathematics revisited: the role of epidemiology in promoting animal health	

SOCIETY FOR VETERINARY EPIDEMIOLOGY AND PREVENTIVE MEDICINE

APPLICATION FOR MEMBERSHIP

Name .....

Address .....

.....

.....

.....

Telephone: .....

Fax: .....

E-mail: .....

Signed ..... Date .....

Please enclose the membership fee (£40 sterling to cover two years' membership) along with this application form. Overseas members without British bank accounts are requested to pay 2 - 3 years in advance. Cheques should be in £ sterling and drawn from a British bank. British members should pay future dues by standing order (forms are available from the Secretary or Treasurer). Payment can also be made by credit card; the appropriate form is available from the Society's website, http://www.svepm.org.uk/, or from the Secretary or Treasurer.

Please send this form to the Society's Treasurer:

Dr David Brodbelt
Royal Veterinary College
Hawkshead Lane
North Mymms
Hertfordshire
AL9 7TA
UK

TEL +44 (0) 1707 667 155
Email: treasurer@svepm.org.uk

Please turn over

INTEREST GROUPS

Please tick appropriate boxes to indicate your interests:

- ☐ Analytical Epidemiology (Observational Studies)
- ☐ Quantitative Epidemiology & Statistical Techniques (Incl. Modelling)
- ☐ Herd/Flock Level Disease Control Strategies
- ☐ National/International Disease Control Policy
- ☐ Sero-Epidemiology
- ☐ Herd Health and Productivity Systems
- ☐ Disease Nomenclature and Epidemiological Terminology
- ☐ Economic Effects of Disease on Animal Production
- ☐ Veterinary Public Health and Food Hygiene
- ☐ Computing, including data logging
- ☐ Computer Programming *per se*
- ☐ Population and Animal Disease Databases
- ☐ Information System Design
- ☐ Geographical Information Systems (GIS)
- ☐ Risk Analysis

CONSTITUTION AND RULES

NAME

1. The society will be named the Society for Veterinary Epidemiology and Preventive Medicine.

OBJECTS

2. The objects of the Society will be to promote veterinary epidemiology and preventive medicine.

MEMBERSHIP

3. Membership will be open to persons either actively engaged or interested in veterinary epidemiology and preventive medicine.

4. Membership is conditional on the return to the Honorary Treasurer of a completed application form and subscription equivalent to the rate for two calendar years at first application or subsequent application following an elapsed subscription. Subsequent annual subscriptions fall due on the first day of May each year.

5. Non-payment of subscription for six months will be interpreted as resignation from the Society.

OFFICERS OF THE SOCIETY

6. The Officers of the Society will be President, Senior Vice-President, Junior Vice-President, Honorary Secretary and Honorary Treasurer. Officers will be elected annually at the Annual General Meeting, with the exception of the President and Senior Vice-President who will assume office. No officer can continue in the same office for longer than six years.

COMMITTEE

7. The Executive Committee of the Society normally will comprise the officers of the Society and not more than five ordinary elected members. However, the Committee will have powers of co-option. Elected officers and ordinary members of the Committee have normal voting rights at committee meetings but co-opted and ex-officio members (e.g. the proceedings editors) do not

ELECTION

8. The election of office bearers and ordinary Committee members will take place at the Annual General Meeting. Ordinary members of the Executive Committee will be elected for a period of three years. Retiring members of the Executive Committee will be eligible for re-election. Members will receive nomination forms with notification of the Annual General Meeting. Completed nomination forms, including the signatures of a proposer, seconder, and the nominee, will be returned to the Secretary at least 21 days before the date of the Annual General Meeting. Unless a nomination is unopposed, election will be by secret ballot at the Annual General Meeting. Only in the event of there being no nomination for any vacant post will the Chairman take nominations at the Annual General Meeting. Tellers will be appointed by unanimous agreement of the Annual General Meeting.

FINANCE

9. An annual subscription will be paid by each member in advance on the first day of May each year. The amount will be decided at the Annual General Meeting and will be decided by a simple majority vote of members present at the Annual General Meeting.

10. The Honorary Treasurer will receive, for the use of the Society, all monies payable to it and from such monies will pay all sums payable by the Society. The Treasurer will keep account of all such receipts and payments in a manner directed by the Executive Committee. All monies received by the Society will be paid into such a bank as may be decided by the Executive Committee of the Society and in the name of the Society. All cheques will be signed by either the Honorary Treasurer or an elected Committee member.

11. Two auditors will be appointed annually by members at the Annual General Meeting. The audited accounts and balance sheet will be circulated to members with the notice concerning the Annual General Meeting and will be presented to the meeting.

MEETINGS

12. Ordinary general meetings of the Society will be held at such a time as the Executive Committee may decide on the recommendations of members. The Annual General Meeting will be held in conjunction with an ordinary general meeting.

GUESTS

13. Members may invite non-members to ordinary general meetings.

PUBLICATION

14. The proceedings of the meetings of the Society will not be reported either in part or in whole without the written permission of the Executive Committee.

15. The Society may produce publications at the discretion of the Executive Committee.

GENERAL

16. All meetings will be convened by notice at least 21 days before the meeting.

17. The President will preside at all general and executive meetings or, in his absence, the Senior Vice-President or, in his absence, the Junior Vice-President or, in his absence, the Honorary Secretary or, in his absence, the Honorary Treasurer. Failing any of these, the members present will elect one of their number to preside as Chairman.

18. The conduct of all business transacted will be under the control of the Chairman, to whom all remarks must be addressed and whose ruling on a point of order, or on the admissibility of an explanation, will be final and will not be open to discussion at the meeting at which it is delivered. However, this rule will not preclude any member from raising any question upon the ruling of the chair by notice of motion.

19. In case of an equal division of votes, the Chairman of the meeting will have a second and casting vote.

20. All members on election will be supplied with a copy of this constitution.

21. No alteration will be made to these rules except by a two-thirds majority of those members voting at an annual general meeting of the Society, and then only if notice of

intention to alter the constitution concerned will have appeared in the notice convening the meeting. A quorum will constitute twenty per cent of members.

22. Any matter not provided for in this constitution will be dealt with at the discretion of the Executive Committee.

Laid down April, 1982
Revised March, 1985; April, 1988; November 1994, March 2014
Corrected January 1997; April 2002